PARASITOLOGY

(Founded by G. H. F. Nuttall, F.R.S.)

EDITED BY

D. KEILIN and P. TATE

IN CONJUNCTION WITH

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LIZARD *FILARIA*: TRANSMISSION BY MOSQUITOES OF *OSWALDOFILARIA CHLAMYDOSAURI* (BREINL) (NEMATODA: FILARIOIDEA)

BY M. J. MACKERRAS

Queensland Institute of Medical Research, Brisbane, Australia

INTRODUCTION

Numerous species of *Filaria* (s.l.) have been recorded from reptiles, but few accounts of their life-histories have been published. Pandit, Pandit & Iyer (1929) observed the development of *Conispiculum guindiensis* in *Culex fatigans* and succeeded in transmitting the infection to clean lizards, and Johnston & Mawson (1923) published an account of the morphology of the developing larvae obtained in preliminary experiments with *Oswaldofilaria chlamydosauri* carried out by the late Dr T. L. Bancroft and the writer using *Culex fatigans* as intermediate host. Recently the opportunity presented itself to carry these experiments a stage further, and full development was obtained in two species of mosquitoes, with transmission to clean lizards.

MATERIALS AND METHODS

The original description of *Oswaldofilaria chlamydosauri* was based on material obtained from the frilled lizard, *Chlamydosaurus kingii* Gray. Frilled lizards are difficult to obtain near Brisbane, but jew lizards, *Amphibolurus barbatus* Cuvier, which harbour the same parasite, are reasonably plentiful and are docile and easy to handle. The lizards used in these experiments were collected near Brisbane or near Inglewood, south Queensland.

At first they were housed in wire cages and kept in the insectary, later they were kept in a small lizard house on the ground, where they were protected from the wind, had more sunlight and better opportunities for exercise. They were fed three times a week on raw meat, and were given live food whenever possible. Water was always present. Jew lizards will not pick up dead meat, but if a strip of it is introduced into the mouth, they will eat it fairly readily. Usually live insects are taken eagerly—small grasshoppers being their favourite insects.

Blood films were obtained by snipping off the tip of the tail, or pricking the foot. It was often difficult to obtain blood from either situation owing to extreme vasoconstriction.

The mosquitoes were bred from larvae collected locally. They were kept in wooden cages measuring $15 \times 11 \times 10$ in., and, after the infecting feed of blood, were fed daily on freshly sliced apple. To obtain infected mosquitoes, a donor lizard was placed in the cage with them and left overnight. Any engorged mosquitoes were removed to another cage next morning.

EXPERIMENTS WITH MOSQUITOES

(1) *Transmission by* Culex fatigans *Wied.*

The donor lizards were four full-grown specimens, nos. 12, 15, 16 and 21, which were collected near Inglewood, S.Q., in April 1950, by Mr J. L. Wassell and made available by courtesy of Professor W. Stephenson, Zoology Department, University of Queensland. On examination each was found to have numerous microfilariae in the blood.

A total of 19 *Culex fatigans* were fed on one or other of these lizards. Of these ten died in less than 4 days, six died 1–3 weeks after feeding and three survived over 4 weeks.

Many of these mosquitoes took up an enormous number of microfilariae, over 190 being counted in one individual. In those mosquitoes which died in less than 4 days the blood was not digested, and microfilariae were found active but still within the gut.

The course of the infection appears to be as follows. The microfilariae lose their sheaths in the midgut of the mosquito, pass through the gut-wall and become loosely coiled up in the fat body. At first they become enormously thicker, sausage-shaped and capable only of sluggish movement. Then they gradually elongate until the infective, slender, active forms develop. These migrate forward and congregate in the head of the mosquito. It is rare to find developing stages in the thorax, but fully developed forms have been observed to escape from the neck when the mosquito was decapitated.

Of the three mosquitoes which survived for 4 weeks, two were heavily infected, and larvae from them were used in an attempt to infect other lizards. These experiments were carried out during winter in an insectary warmed intermittently with a radiator, the temperature ranging from 53 to 87° F. (12°–30° C.).

From the two mosquitoes, dissected on the 29th day, larvae were taken up in 0·4 % NaCl and injected subcutaneously into two lizards, nos. 33 and 35. These were young animals, which had been under observation for 2 months, and in which no microfilariae had been detected.

No. 35 died after 65 days; no microfilariae were

found in the blood, and no developing worms were detected.

No. 33 was examined at approximately monthly intervals for 5 months, during which time no microfilariae were seen. It became very sickly, and in an effort to prolong its life, it was handled only to feed it. It was found dead on the 203rd day from inoculation. Microfilariae were present in the blood, and on dissection six adult females and five adult males were removed from the retroperitoneal tissue and from the floor of the mouth—the normal sites for the adults of this species. These specimens agreed with *Oswaldofilaria chlamydosauri* in all respects.

It may be argued that this lizard could have been infected before capture. However, the long period during which blood films were negative, viz. 2 months before and 5 months after inoculation, would indicate that the infection found at post mortem was most probably derived from the larvae introduced artificially. In the experiment described in the next section, scanty microfilariae appeared in the blood $4\frac{1}{2}$ months after inoculation. It seems probable then that microfilariae would have appeared much earlier in no. 33, had the infection been acquired before capture.

(2) *Transmission by* Culex annulirostris *Skuse*

The donor used in these experiments was no. 12, harbouring numerous microfilariae in the blood. Ten *C. annulirostris* were fed on no. 12. Of these, three died in 1–3 days without digesting the blood, one died at 10 days and two at 23 days, the remainder were killed at 23 days, development of the worms being complete at this stage. This experiment was carried out during summer, the range of temperature being 66–90° F. (19–32° C.).

Those mosquitoes dying during the first 3 days had undigested blood in the midgut; microfilariae were plentiful in this blood, but none was found outside the gut. On the 10th day numerous developing forms were found in the abdominal fat body, none was found in the thorax.

On the 23rd day all mosquitoes were found to be heavily infected with active, fully developed larvae. In one specimen a few embryos, which had not quite reached the infective stage, were also present in the abdomen. The infective-stage larvae were found in the head, proboscis and neck, some at the anterior end of the thorax and very many in the abdominal cavity.

Larvae were taken up in dilute saline as in the previous experiment, and injected into three recipients nos. 36, 38 and 40.

No. 36 had been under observation for 6 months, no. 38 for $4\frac{1}{2}$ months and no. 40 for 4 months before the experiment began. Microfilariae had not been found in the blood of any of them during this period.

Microfilariae appeared in the blood of all three lizards approximately $4\frac{1}{2}$ months after inoculation (128–133 days). They were present in very small numbers at this examination, and one month later their numbers had not increased appreciably. However, at a further examination, 192 days after inoculation, a considerable increase in numbers was noted.

In this experiment there is no absolute proof that these lizards were not infected before capture, but the long period under observation before inoculation (minimum 4 months) and the fact that they all became positive at the same time indicate that their infection was in all probability derived from the larvae inoculated artificially.

Culex annulirostris is a widespread native species and could act as the natural vector of this parasite.

(3) *Experiments with other mosquitoes*

Aedes notoscriptus (Skuse), *Aedes vigilax* (Skuse) and *Anopheles annulipes* Walk. were also given several opportunities to feed on infected lizards. Only *A. notoscriptus* fed, twenty engorged specimens being obtained.

Mortality in this species was very heavy, no less than twelve dying in 1 or 2 days. However, those which succeeded in digesting the blood meal lived well, and one died and seven were killed for dissection between the 20th and 28th day.

One of these harboured twelve, small, sausage-shaped embryos, which in twenty days had only reached the stage found in *Culex fatigans* at 7 days at the same temperature. All the others were free from embryos, so it appears that this species is a poor host.

MORPHOLOGY AND SYSTEMATICS

The morphology of the adults has been adequately dealt with by Breinl (1913), who described the male and also the microfilaria, and by Johnston and Mawson (1943), who added a description of the female. The latter authors described the developing forms in *Culex fatigans*. There is little to add to these descriptions, except to remark that the short, stumpy, microfilaria is sheathed, and that it is usual to see a tiny clump of refractive granules within the sheath at its posterior end.

Developing forms in *C. annulirostris* are indistinguishable from those in *C. fatigans*. The fully developed larvae from the former host measure 1·053–1·161 mm. in length, i.e. they are distinctly smaller than those of *Wuchereria bancrofti*. They are evidently very similar to those of *Conispiculum guindiensis* described by Pandit, Pandit & Iyer

(1929). These authors also noted the prevalence of developing forms in the abdomen of the mosquito, although they also occurred between the thoracic muscles.

As regards taxonomic position, the worm was originally described as *Filaria chlamydosauri* by Breinl (1913), transferred to *Foleyella* by Yorke & Maplestone (1926), and placed in *Oswaldofilaria* Travassos, 1933, by Johnston & Mawson (1943), who pointed out that it differs from *Foleyella* in certain important respects. Following the classification of Wehr (1935) the position may be summarized as follows:

PARASITE: *Oswaldofilaria chlamydosauri* (Breinl). Subfam. Dipetalonematinae, Fam. Dipetalonematidae, Superfam. Filarioidea.

HOSTS: *Chlamydosaurus kingii* Gray and *Amphibolurus barbatus* (Cuvier), Fam. Agamidae.

INTERMEDIATE HOSTS: *Culex annulirostris* Skuse, *Culex fatigans* Wied.

DISTRIBUTION: Queensland: known from Townsville in the North to Inglewood in the south and as far west as Eidsvold. Distribution probably much wider.

SUMMARY

Full development of *Oswaldofilaria chlamydosauri* (Breinl) was obtained in *Culex fatigans* Wied. and *Culex annulirostris* Skuse. Infections in four lizards were observed 4–6 months after inoculation of infective larvae from both species of mosquitoes.

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SOME PARASITIC WORMS FROM FISHES OFF THE COAST OF ICELAND.

I. CESTODA

By GWENDOLEN REES, D.Sc.

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(With 20 Figures in the Text)

In August 1948 a student from this department, Mr J. N. Upton, went on a commercial trawler to the fishing grounds off the coast of Iceland and brought back a collection of parasitic worms from some of the fishes caught in the trawl. Being unable to continue the investigation Mr Upton handed the specimens to the writer for identification and further study. The writer is most grateful for the gift of this collection.

Several records have been made of the helminth parasites of fishes from coastal waters around the British Isles by various writers including Bellingham (1844), T. Scott (1901), A. Scott (1904), Lebour (1908), Nicoll (1915), Woodland (1927), Little (1929), Baylis & Jones (1933), and Rees (1945). There are very few records of parasites from deep-sea fishes taken some distance from the coast. Brief references have been made by Scott (1901) and Nicoll (see Little, 1929) to parasites from deep-sea fishes landed at Aberdeen and by Gallien (1937) to those of deep-water fishes off the coast of Ireland. Rees & Llewellyn (1941) and Rees (1946) recorded parasitic worms from fishes caught in the deep-sea fishing grounds to the west of Ireland, namely, the Irish Atlantic Slope and Porcupine Bank. Records also exist of parasitic worms from fishes of the coastal waters of Europe from the Arctic Sea to the Mediterranean. Most of these are included in Nicoll's (1915) list, although they are found outside British waters. In the present case collections were made off the east coast of Iceland just south of the Arctic Circle. As far as the writer is aware, no previous records exist of the parasites of fishes taken from these waters.

Monogenetic and digenetic trematodes, cestodes and nematodes are present in the collection and these will be dealt with separately. The present paper will be devoted to cestodes and later papers to the remaining groups.

Three species of cestodes were found, namely, *Phyllobothrium acanthiae-vulgaris* (Olsson, 1867), *Echeneibothrium minimum* van Ben. 1850, and the plerocercoid larva of *Hepatoxylon trichiuri* Holten, 1802. Details of their occurrence are shown in Table 1.

The entire collection includes parasitic worms from fishes obtained at depths varying from 58 to 160 fathoms. The fishes concerned here, therefore, are those obtained from the deepest waters, a depth equivalent to that on the Irish Atlantic Slope and parts of the Porcupine Bank.

Table 1

Date	Locality	Depth in fathoms	Host	Parasite
20. viii. 48	14° 58′ W. 63° 20′ N.	115	*Squalus acanthias* L.	*Phyllobothrium acanthiae-vulgaris*
20. viii. 48	10° 54′ W. 65° 55′ N.	160	*Raja clavata* L.	*Echeneibothrium minimum*
20. viii. 48	10° 54′ W. 65° 55′ N.	160	*Gadus callarias* L.	*Hepatoxylon trichiuri*

The worms were fixed on removal from the host in 5% formol. Whole mounts stained with Mayer's paracarmine have been made from some of the specimens, while others have been sectioned, the sections being cut at 5 μ and stained with Ehrlich's haematoxylin and eosin.

Phyllobothrium acanthiae-vulgaris (*Olsson*, 1867)

P. acanthiae-vulgaris was first described by Olsson (1867) under the name of *Phyllobothrideum acanthiae-vulgaris* from *Squalus acanthias* L. in Scandinavian waters. In 1869 he transferred the species to the new genus *Trilocularia* characterized by the possession of four sessile unarmed bothridia, each bothridium having three loculi arranged in a

triangle. The type species of this genus, *T. gracilis*, was described by Olsson (1869) also from *Squalus acanthias*. There seems, however, to be no doubt that these two species are synonymous. According to Southwell (1925), Monticelli, finding numerous scolices and one small strobila of a cestode identical with *Trilocularia gracilis* in *Squalus acanthias*, stated that the genus *Trilocularia* has no justifiable existence but is a synonym of *Monorygma* Diesing. The genus *Monorygma* is characterized by Diesing (1863) as having four sessile bothridia with smooth margins, each with an auxiliary sucker and a terminal myzorhynchus. Monticelli pointed out that the bothridia in Olsson's specimens are not strictly trilocular but consist of a large anterior accessory sucker and a broad bothridium divided incompletely into two parts by a longitudinal septum. On these grounds, therefore, he placed this species in the genus *Monorygma* where it is distinguished from *M. perfectum* and *M. elegans* by the presence of a longitudinal septum in each bothridium. The genus *Monorygma* is now generally considered to be a synonym of *Phyllobothrium*, so the species becomes *P. acanthiae-vulgaris* (Olsson).

Lonnberg (1889, 1890) has recorded the species under the name of *Trilocularia gracilis* from the same host fish, again in Scandinavian waters, and Linton (1924) obtained several specimens over a period of seven years from *Squalus acanthias* off the east coast of North America. It has not been recorded off the coasts of the British Isles, and Joyeux & Baer (1936), though they refer to the species, state that it is unknown off the coast of France. In the present instance eight specimens were obtained from the intestine of *S. acanthias* off the east coast of Iceland.

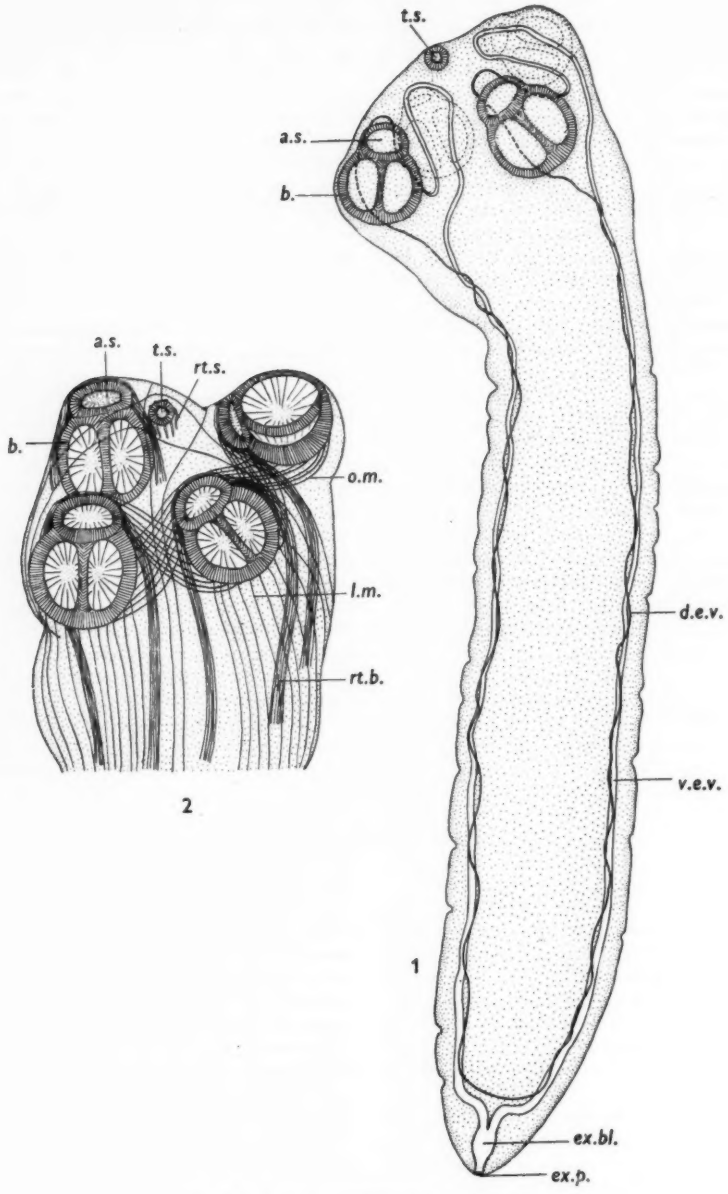
The worm has, apparently, never been found in the sexually mature stage, and existing descriptions of the young stage are extremely brief. An account of it is therefore given below.

External characters. All the specimens, like those previously recorded, were immature, none of them showing any trace of segmentation or rudiments of genitalia. Each consisted of a scolex and unsegmented body measuring 2·90–5·40 mm. in length with a maximum breadth of 0·60–1·07 mm. (Fig. 1.) The scolex is quadrangular in shape, measures 1·00–1·20 mm. in diameter and bears two dorsal and two ventral sessile unarmed bothridia (Figs. 1, 2). The bothridia are oval in outline, they converge anteriorly while their posterior ends stand out somewhat so that they face obliquely forwards. The bothridia at first sight appear to be trilocular, the loculi being arranged in a triangle with the apex anterior. Actually the anterior loculus is an accessory sucker lying immediately in front of the bothridium (Fig. 7). The bothridium is divided into two by a longitudinal septum running down the centre. The bothridia measure 0·29–0·31 mm. in length and 0·35–0·36 mm. in diameter, and the transversely oval accessory sucker 0·12–0·22 mm. in length and 0·21–0·25 mm. in breadth. The wall of the accessory sucker is 0·042 mm. in thickness and of the bothridium 0·053 mm. The anterior face of the scolex is flattened, and sunk in a depression at the apex is a small terminal sucker measuring 0·09–0·17 × 0·10–0·21 mm. and having a depth of 0·07 mm. (Figs. 1–3). The body is covered with cuticle which also lines the bothridia accessory suckers and terminal sucker. Behind the scolex the body tapers gradually to end in a blunt point.

Musculature. The musculature of the body is not well developed. Beneath the cuticle and basement membrane is a layer of very delicate circular muscles followed by a band of longitudinal muscle fibres uniformly arranged and increasing slightly in size as they pass inwards. The fibres are not arranged in bundles (Figs. 3–6). There are no dorso-ventral muscles and no radial muscles. The central region of the body is occupied by the parenchyma, and a fairly broad band of cells is apparent immediately within the longitudinal muscle layer.

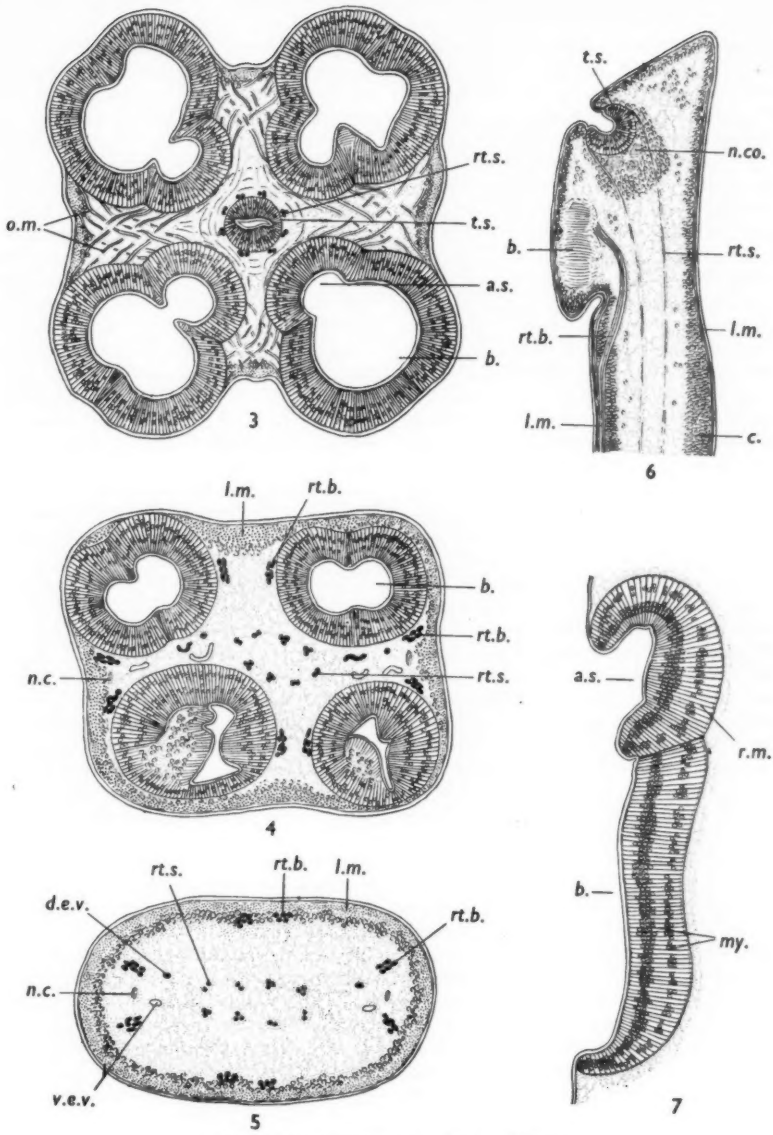
Musculature of scolex. Bothridia, accessory suckers and terminal sucker. The walls of the bothridia, accessory suckers and terminal sucker are composed mainly of radial muscles (Figs. 3, 4, 6 and 7). Below the limiting layer internally and the cuticle externally is a layer of delicate transverse fibres. Large numbers of myoblasts are present among the radial muscles forming roughly two layers, one towards the inner surface and the other towards the outer surface.

Extrinsic muscles of bothridia. Each bothridium is provided with two powerful retractor muscles inserted one on each antero-lateral margin. Some of the fibres continue forward each side around the anterior margin of the accessory sucker where they become continuous with one another (Fig. 2). The retractor muscles pass backwards out of the scolex and into the body of the worm where in transverse section they can be seen as eight bundles of fibres, two dorsally, two ventrally and two on each side (Figs. 4, 5). A short distance behind the scolex the retractor muscles gradually approach the body wall. Laterally they pass one dorsally and one ventrally to the lateral nerves and excretory vessels (Fig. 5). Eventually they all become continuous with the longitudinal muscles of the body wall (Figs. 5, 6). The diameter of the retractor muscles is 0·017–0·02 mm. and they consist of five or six fibres. It is the contraction of these muscles which pulls the anterior end of the bothridium backwards and so allows the forward projection of the posterior margin. A row of longitudinal muscle fibres is also inserted along the posterior border of each bothridium. These are not



Phyllobothrium acanthiae-vulgaris (Olsson)

Fig. 1. Entire worm, dorsal view, showing excretory system.
Fig. 2. Scolex showing extrinsic muscles of the bothridia.



Phyllobothrium acanthiae-vulgaris (Olsson)

Fig. 3. Transverse section through anterior end of scolex showing oblique muscles and the retractor muscles of the terminal sucker.

Fig. 4. Transverse section through posterior end of scolex showing the retractor muscles of the bothridia and of the terminal sucker.

Fig. 5. Transverse section behind scolex showing retractor muscles of bothridia approaching the body wall and the retractor muscles of the terminal sucker still in the central region.

Fig. 6. Sagittal section through terminal sucker and edge of one bothridium showing a retractor muscle of the latter continuous with the longitudinal muscles of the body wall.

Fig. 7. Sagittal section through a bothridium and accessory sucker a little to one side of the middle line.

arranged in bundles, and are again continuous with the longitudinal muscles of the body (Fig. 2). Contraction of these will pull the posterior margin of the bothridium backwards.

A series of oblique muscles connects each bothridium and its accessory sucker with the one next to it on each side all around the scolex (Figs. 2, 3). The oblique muscles pass in each case from the lateral margin of the accessory sucker and anterior half of the bothridium obliquely backwards to the lateral margin of the next bothridium. This occurs on both margins of the bothridia. There is therefore a crossing of the oblique muscle fibres in each interbothridial space dorsally, ventrally and laterally. The oblique muscles are more superficially placed than the retractor muscles. Their contraction will narrow the diameter of the scolex and approximate the bothridia.

Extrinsic muscles of terminal sucker. The terminal sucker is also provided with extrinsic muscles in the form of eight retractors which are inserted in a ring around the outer margin, two dorsally, two ventrally and two on each side (Figs. 2, 3). These continue backwards through the central region of the scolex (Fig. 4) to about half the length of the body (Figs. 5, 6) before they eventually diverge towards the body wall to become continuous with the longitudinal muscles. These retractors are more delicate than those of the bothridia. They measure 0·013 mm. in diameter and consist each of three or four fibres. Their contraction will presumably withdraw the terminal sucker deeper into its cavity.

Those longitudinal muscles of the body not concerned with the bothridia continue forwards through the scolex. At the apex the dorsal and ventral fibres become continuous with one another and likewise the lateral fibres. They cross over at the apex, being deflected slightly to pass around the terminal sucker (Figs. 2, 3).

Excretory system. A dorsal and a ventral excretory vessel extend along the length of the body on each side, the ventral being slightly greater in diameter than the dorsal (Fig. 1). The two dorsal vessels are connected with one another at the posterior extremity just in front of the excretory bladder. Otherwise there is no connexion between the vessels of each side. In the scolex the ventral vessels extend forwards on the inner side of the corresponding bothridium as far as, or a little beyond, the accessory sucker where they turn back and pass to the posterior margin of the bothridium, thus forming a loop. The dorsal vessels behave in the same way. On each side of the scolex, between the dorsal and ventral bothridia, both vessels are continuous with one another. At the posterior extremity of the body the two ventral vessels unite to form an elongated excretory bladder measuring 2·00 × 0·02 mm. which opens to the exterior by a small terminal pore.

Nervous system. A lateral nerve cord is present on each side of the body. The details of the nervous system of the scolex could not be traced in detail but three transverse commissures, two anterior and one posterior, pass transversely across the scolex immediately behind the terminal sucker (Fig. 6).

Echeneibothrium minimum *Van Beneden*, 1850

The identification of this species has presented some difficulties. While the characters of the genus *Echeneibothrium* van Ben. are well defined, the individuals are so variable that it is almost impossible to separate them into well-defined species. According to Beauchamp (1905): 'When the question of species arises difficulties begin, and, whoever has seen living examples of these animals will understand that a specification is practically impossible (see van Beneden's drawings). Neither the number of loculi, which is perhaps fairly constant (but regarding which mistakes can be easily made...) nor the general shape of the bothridia, nor the presence or absence of a myzorhynchus (on which Linton has founded his genus *Rhinebothrium*) are characters above criticism. Finally, the length of the neck and the law of the growth of the segments are... characteristics of a variety, or of an individual, rather than a species. It thus follows that in the genus there is, perhaps, not a single species which can be clearly differentiated from the others.'

Using the key given by Joyeux & Baer (1936) the present specimens can be identified as *Echeneibothrium dubium* van Ben. 1858. Southwell (1925), however, considers *E. dubium* to be a synonym of *E. minimum*. Another species containing a large number of very variable forms is *E. tumidulum* (Rud. 1819) (= *E. variabile* van Ben. 1850). According to Beauchamp (1905) 'certain authors have united under the name *E. tumidulum* the *E. variabile* and *E. minimum* of van Beneden, which nevertheless seem fairly distinct'. The chief distinctions between *E. tumidulum* and *E. minimum* are the very large myzorhynchus in the former and the fact that the bothridia are divided into loculi by numerous transverse and a single longitudinal septum. In *E. minimum* there is no longitudinal septum, the bothridia being divided by a series of transverse loculi only, and the myzorhynchus is smaller, being narrower in diameter than the neck. This being so, the present specimens cannot be included in *E. tumidulum* but fall rather into the species *E. minimum*.

The specimens resemble to some extent *E. affine* which is described by Olsson (1867) as having 'Long, pedunculated, mobile bothridia with three (or more?) transverse septa, a long cylindrical and retractile myzorhynchus, furnished with a definite tubular apex. Neck long... last segments oblong;

genital pores marginal, alternate; cirrus spiny... the head does not agree in all details with that of *E. dubium* nor with *E. variabile*....The bothridia, with fairly long peduncles, often have the appearance of a barrel-shaped flower vase... are sometimes egg shaped or like a concave leaf etc.' Southwell (1925) considers *E. affine* to be synonymous with *E. tumidulum* (= *E. variabile*) in spite of the absence of longitudinal septa in the bothridia, while other writers consider it to be the same as *E. minimum*. The myzorhynchus, however, in *E. affine* is three times as thick at the extremity as at the base, a character of *E. tumidulum*; this is not so in the present specimens where it is broadest at the base and tapers slightly to the extremity.

It can be seen, therefore, that identification of the species within the genus *Echeneibothrium* is extremely difficult, and in the present state of our knowledge of the members of this genus there seems to be no alternative but to include the present form in the species *E. minimum* van Ben. (1850) (= *E. dubium* van Ben. (1858), *E. sphaerocephalum* Diesing (1863), *E. trygonis* Shipley & Hornell (1906), *Tiarabothrium javanicum* Shipley & Hornell (1906), *Rhinebothrium shipleyi* Southwell (1911)).

Echeneibothrium minimum was first described by van Beneden (1850) from *Trygon pastinaca* off the coast of Belgium and was again found by him (1858) in *Raja batis* from the same locality. Since then there have been several records of the species from various parts of the world. Lonnberg (1889, 1890) found it in *Raja clavata*, *R. batis*, *R. radiata* and *R. vomer* off the west coast of Norway. Shipley & Hornell (1906) recorded it from *Rhinoptera javanica*, *Dasybatus walga* and *D. kuhli* off the coast of Ceylon, and Southwell (1911, 1930) from *D. kuhli* in the same neighbourhood and from *Carcharias* sp. in Indian waters. Linton (1924) recorded it from *Raja eglanteria* and *R. stabuliforis* at Wood's Hole, and Leon-Borcea (1934) from *Trygon pastinaca* in the Black Sea. It has been found in the English Channel by Joyeux & Baer (1936) in *T. pastinaca*, *Raja clavata*, *R. batis* and *R. punctata*. Otherwise there is no existing record of it from fishes around the coasts of the British Isles and none from the coast of Iceland where now two specimens have been recovered from the spiral valve of *R. clavata*.

In view of the extreme variability of species included in the genus *Echeneibothrium* a description of the present form is given below.

External characters. Of the two specimens obtained, the longer measured 18·0 mm. in length and consisted of a scolex and 157 segments. Both specimens were incomplete and in neither were there any fully mature or gravid segments. The maximum breadth of the strobila near the posterior extremity was 0·58 mm.

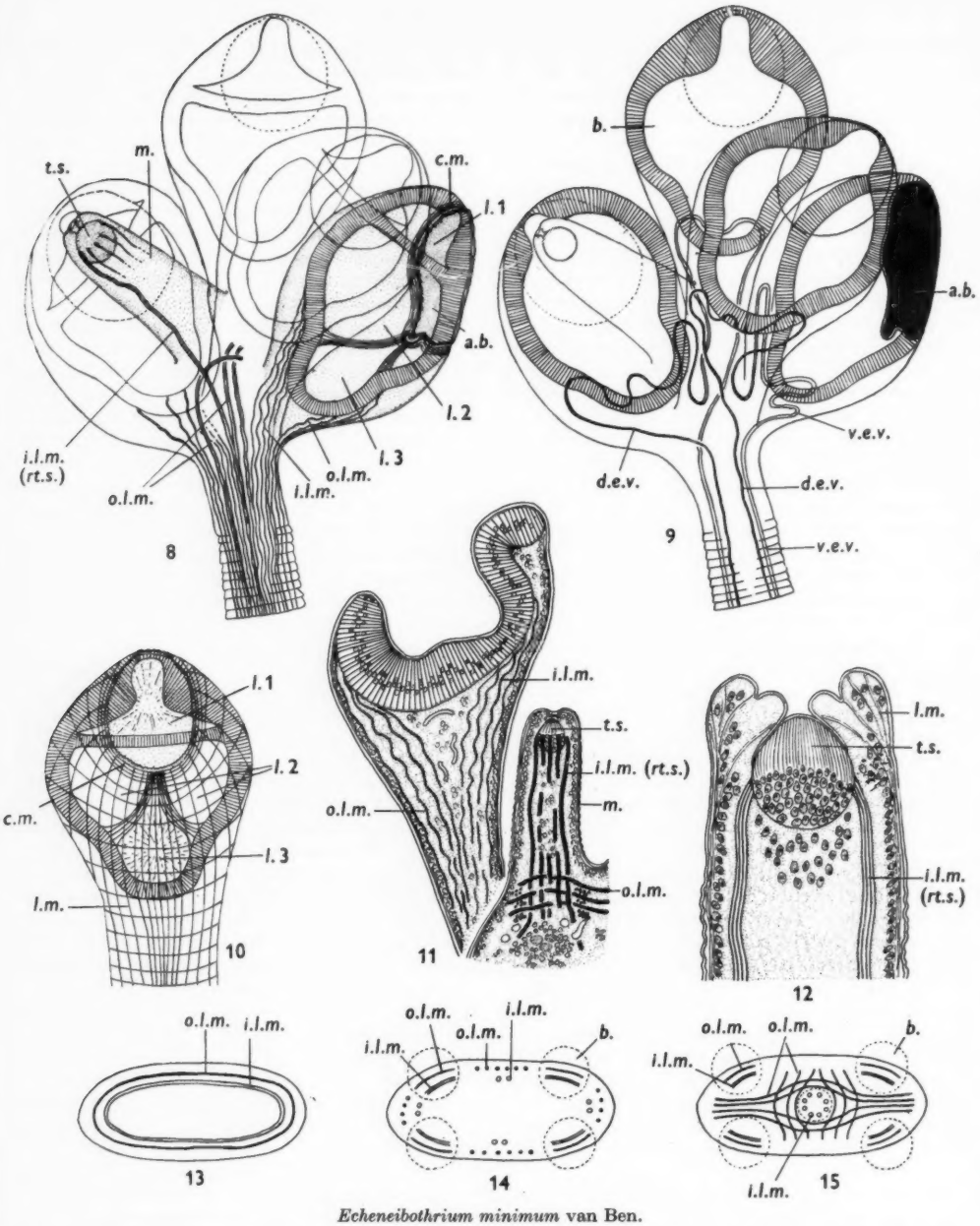
The scolex bears two dorsal and two ventral pedunculate bothridia (Figs. 8, 9). The length of the bothridium and peduncle is 0·50 mm. The bothridium is oval or somewhat barrel-like in shape with a maximum diameter of 0·32 mm. The opening is situated subterminally on the outer face and surrounded by a narrow fold of cuticle (Figs. 8, 10). The cavity of the bothridium is deep, narrowing at the base and is divided somewhat indistinctly into three loculi (Figs. 8, 10). A transverse septum is present on the inner wall of the cavity just behind the apex and there are two oblique septa on the outer wall. These latter extend from the posterior margin of the aperture backwards and outwards on each side passing around to the inner wall. There are therefore three loculi in each bothridium, two on the inner face one behind the other and one on the outer face. The loculi, however, are not always well marked, the prominence of the septa depending on the degree of expansion of the bothridia. There is no median longitudinal septum.

At the apex of the scolex is a long mobile myzorhynchus (Figs. 8, 9 and 11) measuring 0·25 mm. in length and 0·12 mm. in breadth at the base. At the extremity of the myzorhynchus is a sucker measuring 0·05 mm. in diameter sunk in a depression which opens terminally and has a double rim around the margin.

The scolex is followed by a short neck 0·21 mm. long and 0·13 mm. wide. The young segments are broader than long but as the male organs develop the segments become almost square in outline, measuring 0·19 mm. long and 0·11 mm. broad. Further back the breadth increases at a greater rate than the length, and by the time the male organs are almost fully developed the segments measure 0·27 mm. long and 0·58 mm. broad. In neither strobila were the female organs fully developed, but it was possible to find all the constituent parts in sections. Only in the three last segments of the longer specimen was the genital aperture fully formed and the cirrus evaginated. The genital apertures alternate irregularly and are situated a little behind the middle of the lateral margin. As no gravid segments were present their shape cannot be described.

Musculature. The superficial musculature consists of delicate outer circular and inner longitudinal layers lying immediately below the basement membrane. The deep musculature consists of longitudinal muscles only, which are arranged in bundles forming a broad band surrounding the medullary region throughout the length of the strobila (Figs. 17–20). There is no essential change in the neck region except that the bundles extend more deeply into the central region from whence they continue into the scolex.

Musculature of scolex. The muscles of the scolex and the extrinsic muscles of the bothridia and



Echeneibothrium minimum van Ben.

Fig. 8. Scolex showing loculi of one bothridium and part of the musculature.

Fig. 9. Scolex showing excretory system.

Fig. 10. Single bothridium, posterior view, showing loculi and superficial musculature.

Fig. 11. Longitudinal section through one bothridium and myzorhynchus showing musculature.

Fig. 12. Longitudinal section through extremity of myzorhynchus showing terminal sucker and muscles.

Figs. 13–15. Diagrams illustrating behaviour of deep musculature on entering the scolex.

myzorhynchus are all derived from the muscles entering the scolex from the neck. Each bothridium is provided with superficial muscles continuous with those of the strobila (Fig. 10). The circular fibres surround the peduncle and continue on to the bothridium. Around the opening of the bothridium they are slightly more concentrated, forming a kind of sphincter (Figs. 8, 10), the contraction and relaxation of which control the size of the aperture. Superficial circular muscles are also present in the wall of the myzorhynchus. The superficial longitudinal muscles are better developed in the scolex than in the strobila. On entering the scolex they form small bundles which extend up the outer sides of the bothridia to be inserted around the margin of the aperture in each case. Those inserted into the posterior half of the margin are the better developed (Fig. 10). Contraction of these muscles will enlarge the aperture of the bothridium. Superficial longitudinal muscles are also present on the anterior side of each bothridium. Some of these pass diagonally from the right dorsal to the left ventral bothridium and vice versa, others pass between neighbouring bothridia dorsally, laterally and ventrally. At the apex of the scolex some of the diagonal muscles coming from all four bothridia continue into the myzorhynchus and form a layer below the superficial circular muscles. At the apex of the myzorhynchus these fibres spread out and are inserted into the double lip around the terminal aperture (Fig. 12).

The deep longitudinal muscles on entering the scolex divide into two layers one inside the other (Fig. 13). Dorso-laterally and ventro-laterally the fibres of both layers pass into the peduncles of the bothridia (Fig. 14). Those of the outer layer are inserted into the posterior part of the outer side of the wall of the bothridium and those of the inner layer into the posterior part of the inner side (Figs. 8, 11). Mid-dorsally, mid-ventrally and laterally the fibres of the outer layer extend forwards into the scolex between the bothridia (Fig. 14). At the apex the outer dorsal and ventral muscles become continuous with one another and likewise the outer muscles from each side. None of these enters the myzorhynchus (Figs. 8, 11 and 15). The mid-dorsal, mid-ventral and lateral fibres of the inner layer combine to form eight bundles, two dorsal, two ventral and two on each side, which pass towards the centre of the scolex and enter the myzorhynchus (Figs. 8, 11, 12 and 15). They continue along the myzorhynchus and are inserted in a ring into the terminal sucker at about half way along its length. These are the retractor muscles of the terminal sucker. Each has a diameter of 0·008 mm.

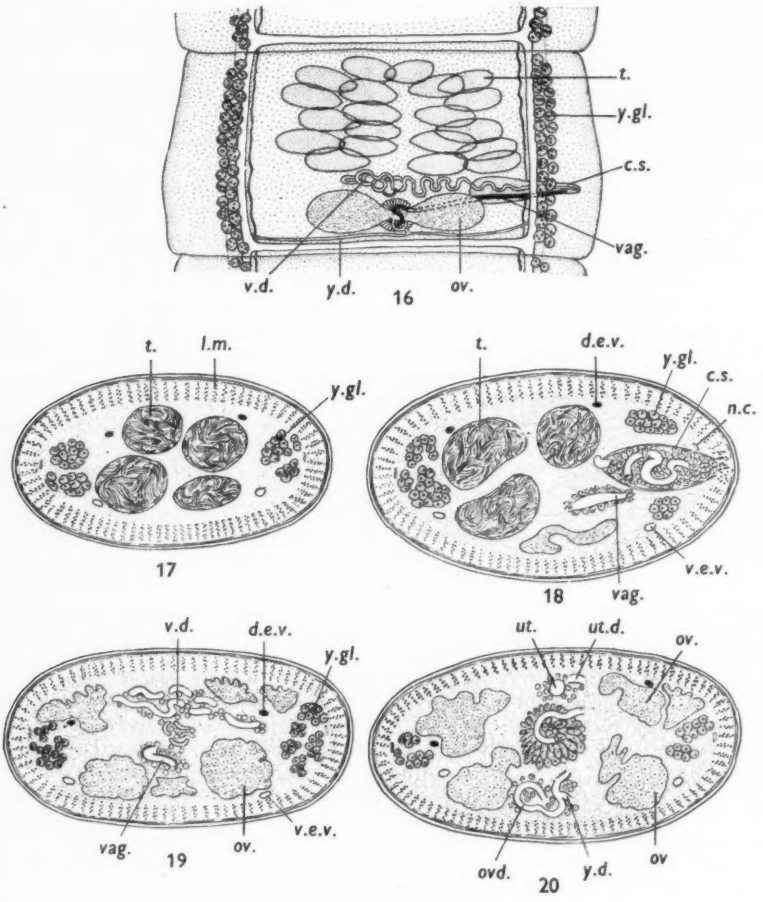
The muscular walls of the bothridia consist mainly of radial fibres with delicate circular and longitudinal fibres on the inner and outer faces. The wall measures 0·053 mm. in thickness at its widest point which is near the anterior apex of the bothridium (Figs. 8–10). Elsewhere, the wall is thinner, measuring 0·021 mm. The terminal sucker of the myzorhynchus consists of a mass of radial muscles placed like a cushion in the apical depression. A mass of cells, possibly gland cells, occupy the proximal half. Myoblasts are present among the radial muscles of the bothridial wall.

Excretory system. A dorsal and a ventral excretory vessel extend throughout the strobila on each side (Figs. 9, 16). They are most conspicuous in the neck and immature regions. The dorsal vessel is slightly smaller than the ventral. The arrangement in the scolex is a very simple one (Fig. 9). The dorsal vessels extend into the peduncles of the dorsal bothridia and the ventral into the peduncles of the ventral bothridia. In each case they describe several loops in passing up to the apex of the peduncle where they turn back, the two dorsal vessels turn ventrally and the two ventral dorsally. At the bases of the peduncles they meet, and on each side the dorsal and ventral vessels become continuous with one another. No anastomosis has been observed between any of the vessels and no vessels have been found in the myzorhynchus. In the strobila a transverse vessel connects the two ventral vessels in each segment, but these are not easy to distinguish (Fig. 16).

Genital system. In the specimens available only the male organs were fully developed. It was, however, possible to make out most of the constituent parts of the female genitalia.

Male genitalia. There are twenty testes arranged in two fields almost meeting in the middle line, occupying about two-thirds of the length of the proglottid and not extending beyond the ovary (Fig. 16). They are large and transversely oval, measuring 0·04 × 0·08–0·10 mm. In transverse section each testis in its widest part can be seen to occupy about half the depth of the medullary region (Figs. 17, 18). The vas deferens is very long and coiled, and occupies about the middle third of the width of the segment towards the dorsal side behind the testes and dorsal to the anterior margin of the ovary (Figs. 16, 19). The cirrus sac, which, in the specimen sectioned had not yet acquired an opening to the exterior, measured 0·18 mm. long with a maximum breadth of 0·064 mm. It passes between the dorsal and ventral excretory vessels and ventral to the lateral nerve (Figs. 16, 18). No vesicula seminalis was apparent and there were, as yet, no sperms in the vas deferens. In the last two segments of one mounted specimen the cirrus was evaginated but unfortunately was torn away from the body wall. The evaginated cirrus measures 0·327 mm. in length and 0·027 mm. in breadth and is covered with spines.

Female genitalia. The ovary is situated posteriorly lying behind the coils of the vas deferens and towards the ventral side of the segment (Figs. 16, 19 and 20). It is bilobed in surface view and X-shaped in transverse section, occupying the whole depth of the medullary region on each side. The oviduct arises from the posterior side of the median portion of the ovary (Fig. 16). From here it passes ventrally for a short distance then turns dorsally passing behind the median portion of the ovary where it turns dorsally to open into the dorsal side of the uterus which is as yet very poorly developed, consisting of a small almost spherical sac (Fig. 20). Opening into the oviduct shortly after its origin is the vagina (Fig. 16). The opening of the vagina to the exterior was not present at this early stage but the developing



Echeneibothrium minimum van Ben.

Fig. 16. Mature proglottid, ventral view.
Fig. 17. Transverse section through anterior region of proglottid showing testes.
Fig. 18. Transverse section showing cirrus sac, vagina and part of ovary.
Fig. 19. Transverse section showing vas deferens, vagina and ovary.
Fig. 20. Transverse section showing ovary, oviduct, Mehlis's gland, uterine duct and uterus.

vagina runs along the ventral side of the cirrus sac (Fig. 18). It passes towards the centre of the segment where it describes a loop just anterior to Mehlis's gland and ventral to the vas deferens before it opens into the oviduct (Fig. 19). The follicular yolk cells are situated laterally and extend along the length of the proglottid just outside the excretory vessels (Figs. 16–20). From the lateral yolk ducts transverse ducts pass across the posterior end of the segment and unite to form a median yolk duct which opens into the oviduct some distance beyond the opening of the vagina (Fig. 16).

As already mentioned, none of the specimens was fully mature but it was possible to recognize most of the constituent parts of the reproductive organs. No gravid segments were present, so the form and dimensions of the uterus and eggs could not be observed.

Plerocercoid larva of Hepatoxylon trichiuri *Holten*, 1802

This species is perhaps better known under the name of *Dibothriorhynchus grossum* (Rud. 1819), but according to Dollfus (1942) the name *Hepatoxylon trichiuri* Holten 1802 takes precedence. Only one specimen was obtained, from the coelome of *Gadus callarias* L., off the coast of Iceland. The species has been recorded from time to time from a large number of hosts and seems to have a world-wide distribution. Some of the localities from which it has been recorded are California, the Atlantic coast of the United States and Canada, Brazil, Valparaiso, Portugal, France, Belgium, Naples, Africa, the Pacific and Japan. According to Dollfus (1942), it has been found on several occasions around the coasts of the British Isles. He stated that Templeton (1836), Drummond (1838) and Bellingham (1844) recorded it from *Salmo salar* off the Irish coast; Band, in 1853, from *S. selar* and *S. trutta* at Berwick on Tweed; Tosh (1905) from the same hosts and locality; Johnstone (1912) from *Gadus virens* at St Kilda, and Heitz (1917) from *Salmo trutta* off the Irish coast. Rees & Llewellyn (1941) record it from *Gadus virens* caught in the deep sea fishing grounds to the west of Ireland, the exact locality being 54° N. and 11° 11′ W. and the depth 120–150 fathoms. In the present case the locality is 11° 55′ farther north and 0° 16′ farther west and the depth 160 fathoms. A comprehensive list of the hosts and localities from which the species has been obtained in various parts of the world is given by Dollfus (1942), and a full description of the anatomy has already been published by the writer (Rees, 1941).

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Key to lettering of figures

(All drawings are semi-diagrammatic)

a.b.	aperture of bothridium	*o.l.m.*	outer longitudinal muscles
a.s.	accessory sucker	*o.m.*	oblique muscles
b.	bothridium	*ov.*	ovary
c.	cells below muscle layer	*ovd.*	oviduct
c.m.	circular muscles	*r.m.*	radial muscles
c.s.	cirrus sac	*rt.b.*	retractor muscles of bothridium
d.e.v.	dorsal excretory vessel	*rt.s.*	retractor muscles of terminal sucker
ex.bl.	excretory bladder	*t.*	testis
ex.p.	excretory pore	*t.s.*	terminal sucker
i.l.m.	inner longitudinal muscles	*ut.*	uterus
l. 1, 2 and 3.	loculi of bothridium	*ut.d.*	uterine duct
l.m.	longitudinal muscles	*v.d.*	vas deferens
m.	myzorhynchus	*v.e.v.*	ventral excretory vessel
my.	myoblasts	*vag.*	vagina
n.c.	lateral nerve cord	*y.d.*	yolk duct
n.co.	nerve commissure	*y.gl.*	yolk glands

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SOME PARASITIC WORMS FROM FISHES OFF THE COAST OF ICELAND

II. TREMATODA (DIGENEA)

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(With 2 Figures in the Text)

The present paper is concerned with the digenetic trematodes in a collection of parasitic worms obtained in August 1948 from fishes off the coast of Iceland. The cestodes from the same collection have already been dealt with (Rees, 1953).

There are many records of digenetic trematodes from marine fishes in various parts of the world. The trematodes of British marine fishes are probably better known than those of any other region due to the work of Lebour (1908), Johnstone (1909), Nicoll (1914, 1915), Baylis & Jones (1933) and others. Nicoll (1915) compiled a list of trematodes from marine fishes which included seventy-three genera and 157 species of Digenea. A number of studies has also been made from other European waters. Van Beneden (1858) was one of the earliest workers in this field, making collections along the Belgian coast. Olsson (1868) recorded twenty-seven species of Digenea from Scandinavian fishes, and Levinsen (1881) studied the trematodes of Arctic fishes off the west coast of Greenland. One of the most important papers on trematodes of fishes is that of Odhner (1905) who recorded nineteen species of Digenea from Arctic waters. Parasites of Mediterranean fishes have been investigated by Rudolphi, Loos and others.

Later the study of fish trematodes extended to Canadian and American waters. Stafford (1904) recorded thirty-seven trematodes from Canadian fishes, and further work has been done on the subject by Miller (1941) and Heller (1949). In America the work of Linton (1911) at Wood's Hole and Tortugas has been extended by Manter (1926 and 1934).

Further contributions have been made in this field in various other parts of the world: in Australia by Woolcock (1935), in India by Chauhan (1945), in Ceylon by Lühe (1906) and in Japan by Yamaguti (1934).

As far as the writer is aware only one species of digenetic trematode has previously been recorded off the coast of Iceland, no systematic study having been made of the parasites of fishes from Icelandic waters.

In the present instance eight species of Digenea were found, seven of which are already known and the remaining one is new. Although this collection is not large it provides evidence of a wider geographical distribution of parasites which have been previously described from other regions of the world and of some new hosts for the known species.

Specimens had already been fixed, in 5 % formol, when handed to the writer. Whole mounts, stained with Coelestin blue B or Mayer's paracarmine, have been made from these.

A list of fishes examined, with their digenetic trematodes, is given in Table 1 and a classified list of the trematodes with their hosts in Table 2. The species are then dealt with in turn, details of the host, the exact locality and depth from which the fishes were taken, being given, together with a brief account of each species. The new species is described as fully as the material would allow.

(1) **Fellodistomum fellis** (Olsson, 1867)

Synonymy: *Distoma fellis* Olsson, 1868; '*Distomum fellis* Olsson, Stossich, 1886; *D. fellis* Olsson, Jacoby, 1900; *Fellodistomum incisum* (Rud. 1809), Stafford 1904; *F. agnotum* Nicoll, 1909

Host: *Anarhichas lupus* L.; locality: east coast of Iceland, 12° 33′ W., 66° 27′ N.; depth: 65 fathoms; incidence: 17 August 1948, three specimens; habitat: gall bladder.

Fellodistomum fellis (Olsson) is a very common parasite of *Anarhichas lupus* L., the wolf-fish or cat-fish. According to Nicoll (1909) it occurs in almost every specimen examined and at all ages. As far as the writer is aware it does not occur in any other host species. It has not been recorded previously off the coast of Iceland, but has been reported from Scandinavian waters by Olsson (1868), from the east coast of Britain by Lebour (1908) and Nicoll (1915), and from the east coast of Canada, namely, off New Brunswick, Nova Scotia, and Prince Edward Island by Stafford (1904).

Fellodistomum fellis was first described by Olsson (1868) and later by Stossich (1886) and Jacoby (1900). Rudolphi described two digenetic trematodes from *Anarhichas lupus*; one *Distomum incisum*, synonymous with *Fellodistomum fellis*, Stafford retaining the former name as the type species. Jacoby (1900) and Nicoll (1909), on the other hand, consider them to be distinct species. Because of

Table 1. *List of fishes with their digenetic trematodes*

Class:	Selachii	
Subclass:	Euselachii	
Order:	Hypotremata	
Family:	Rajidae	
	Raja clavata L.	*Otodistomum veliporum* (Creplin)
Subclass:	Neopterygii	
Order:	Anacanthini	
Family:	Gadidae	
	Molva byrkelange Walbaum	*Gonocerca crassa* Manter
	Brosme brosme (Müll.)	*Derogenes varicus* (Müll.)
Order:	Percomorphi	
Family:	Anarhichadidae	
	Anarhichas lupus L.	*Fellodistomum fellis* (Olsson)
		Plagioporus idonea (Nicoll)
		Lepidophyllum steenstrupi Odhner
	Anarhichas minor Olafsen	*Steganoderma pycnorganum* n.sp.
Order:	Scleroparei	
Family:	Scorpaenidae	
	Sebastes marinus (L.)	*Hemiurus communis* Odher
Order:	Heterosomata	
Family:	Pleuronectidae	
	Platichthys flesus (L.)	*Derogenes varicus* (Müll.)

Table 2. *List of digenetic trematodes with their fish hosts*

Family:	Fellodistomatidea, Odhner	
Subfamily:	Fellodistomatinae, Nicoll	
(1)	*Fellodistomum fellis* (Olsson)	*Anarhichas lupus* L.
Family:	Allocreadiidae, Stossich	
Subfamily:	Allocreadiinae, Looss	
(2)	*Plagioporus idonea* (Nicoll)	*Anarhichas lupus* L.
Family:	Azygiidae, Odhner	
(3)	*Otodistomum veliporum* (Creplin)	*Raja clavata* L.
Family:	Hemiuridae, Lühe	
Subfamily :	Derogenetinae, Odhner	
(4)	*Derogenes varicus* (Müll)	*Platichthys flesus* (L.)
		Brosme brosme (Müll)
(5)	*Gonocerca crassa* Manter	*Molva byrkelange* Walbaum
Subfamily:	Hemiurinae Looss	
(6)	*Hemiurus communis* Odhner	*Sebastes marinus* (L.)
Family:	Zoogonidae, Odhner	
Subfamily:	Lecithostaphylinae, Odhner	
(7)	*Lepidophyllum steenstrupi* Odhner	*Anarhichas lupus* L.
(8)	*Steganoderma pycnorganum* n.sp.	*Anarhichas minor* Olafsen

from the gall bladder, and the other, which he named provisionally *D. anarrhichae lupi*, from the stomach. There is no doubt that the latter is synonymous with *Fellodistomum fellis*, which name now stands as that given by Rudolphi was provisional. Van Beneden (1871), Stossich (1886), Stafford (1904) and Miller (1941) consider *Distomum incisum*, also, as being Stafford's description stating that the ovary is on the left side of the body in *Distomum incisum*, Nicoll doubted whether he was dealing with *Fellodistomum fellis*. According to Miller (1941), however, the specimen in Stafford's collection shows the ovary on the right side. There is therefore little doubt that the two are synonymous.

Dawes (1946) is of the opinion that *F. agnotum* Nicoll, also, is synonymous with *F. fellis*. Nicoll (1909) stated that the former occurs in the duodenum, whereas *F. fellis* is confined to the gall bladder. But if *Distomum anarrhichae lupi* can occur in the stomach, the difference in location cannot be of vital importance. The chief features of *Fellodistomum agnotum* are, according to Nicoll, a more elongated body, the smaller size of the ventral as compared with the oral sucker, the more anterior yolk glands and the backward prolongation of the uterus. No other record exists of this species, and Dawes considers that the two are synonymous.

The diagnostic features of *F. fellis* may be summarized as follows. Dull green when fresh with pinkish suckers; 2·5–3·3 mm. long, 1·1–1·5 mm. broad; cuticle smooth, wrinkled anteriorly; suckers globular, ventral sucker slightly behind the middle of the body and more than twice as large as the oral sucker; prepharynx short, pharynx large, oesophagus very short, caeca extending as far as the testes; excretory vesicle Y-shaped with short stem;

Table 3

	(1)	(2)	(3)
Length	2·90	3·00	3·20
Breadth	1·10	1·60	1·80
Oral sucker	0·40	0·45	0·40
Ventral sucker	1·10	1·50	0·90
Pharynx	0·21	0·21	0·18
Eggs	0·043 × 0·024	0·042 × 0·023	0·042 × 0·023

genital pore on large papilla in front of the anterior margin of the ventral sucker to the left of the middle line; cirrus-sac contains ductus ejaculatorius, pars prostatica and bipartite vesicula seminalis; testes oval, side by side, between ventral sucker and posterior extremity; ovary slightly lobed and in front of the right testis; vitellaria lateral in front of ventral sucker; uterus with descending and ascending limbs; eggs about 0·042 × 0·023 mm.

Several adequate descriptions of *F. fellis* have been published and so no further account of it will be given here. The three specimens obtained in the present investigation have been measured and some of the measurements, in mm., are given in Table 3. As they were taken from specimens which had been flattened, the dimensions are slightly greater than those previously recorded.

(2) **Plagioporus idonea** (Nicoll, 1909)

Synonymy: *Lebouria idonea* Nicoll, 1909; *L.* (*Lebouria*) *idonea* Issaitschikow, 1928

Host: *Anarhichas lupus* L.; locality: east coast of Iceland, 12° 33′ W., 66° 27′ N.; depth: 65 fathoms; incidence: 17 August 1948, one specimen; habitat: intestine.

Plagioporus idonea was first described by Nicoll (1909) as *Lebouria idonea* from the intestine and occasionally the stomach of *Anarhichas lupus* L. The genus *Plagioporus* was proposed by Stafford (1904), and four species of the genus have been recorded by Nicoll (1915), three from British waters, namely, *P. idonea* from *Anarhichas lupus* L. and *A. latifrons* Steenstrup & Hallgrimsson, *Plagioporus varia* (Nicoll) from *Collionymus lyra* L. and *Pleuronectes platessa* L. and *Plagioporus alacris* (Looss) from *Labrax mixtus* L., *L. bergylta* Ascanius, *Crenilabrus melops* (L.), *Ctenolabrus rupestris* (L.) and *C. exoletus* (L.) and one, namely, *Plagioporus tumidulum* (Rud.) from *Entelurus aequoreus* (L.) and *Syngnathus acus* L. in European waters.

According to Dawes (1946) Issaitschikow proposed the erection of three subgenera 'an apparently unnecessary procedure as regards British species'.

So far *Plagioporus idonea* has been recorded only off the east coast of Britain by Lebour (1908) and Nicoll (1915). According to Nicoll (1915) the specimen of *P. idonea* recorded by Johnstone (1910) from *Callionymus lyra* L. in Wolverston Channel, Morecambe Bay, should have been identified as *Plagioporus varia*. Other species of the genus have been recorded from Canadian waters by Stafford (1904) and from the Russian Arctic by Issaitschikow. In the present instance *P. idonea* has been recorded for the first time off the coast of Iceland.

The diagnostic features of *P. idonea* are as follows. Yellowish in colour when fresh; 1·5–2·5 mm. long, 0·7–1·0 mm. broad, slightly attenuated anteriorly; cuticle smooth; ventral sucker nearly in middle of body, transversely oval and about twice the size of the oral sucker; prepharynx wide, pharynx large, oesophagus about equal in length to pharynx, caeca extending to posterior extremity; genital pore median midway between suckers, sometimes to the left or right of the middle line; cirrus sac small; vesicula seminalis much convoluted, no well-differentiated pars prostatica; testes one behind the other midway between ventral sucker and posterior extremity; ovary immediately in front of and to the right of the anterior testis; large receptaculum seminis dorsal to and in front of ovary; vitellaria lateral extending from the pharynx to the posterior end uniting behind the testes; uterus confined to the area between the ovary and the posterior border of the ventral sucker; eggs 0·0715–0·0746 × 0·039–0·0435 mm.

No further description of *P. idonea* will be given here as only one specimen was available. The measurements, in mm., taken from this specimen are as follows: length, 2·80; breadth, 1·10; oral sucker, 0·28; ventral sucker, 0·60; pharynx, 0·21 × 0·26; eggs, 0·07 × 0·04 mm.

(3) **Otodistomum veliporum** (Creplin, 1837)

Synonymy: *Distoma veliporum* Creplin, 1837; *D. insigne* Diesing, 1850; *Fasciola squali grisei* Risso, of Diesing, 1850; *Distoma microcephalum* Band, 1853; *D. cestoides* Beneden, 1870; *D. nigrescens* Olsson, 1876; *Agamodistomum chimerae* Ariola, 1899; *Xenodistomum melanocystis* Stafford, 1904; *Otodistomum cestoides* Beneden, of Odhner, 1911; *Cercaria cestoides* Nicoll, 1913; *Otodistomum cestoides* Dollfus, 1937; *O. c. pacificum* Dollfus, 1937; *O. veliporum leptotheca* Dollfus, 1937; *O. v. veliporum* Dollfus, 1937; *O. v. pachytheca* Dollfus, 1937; and probably *Otodistomum pristiophori* (Johnstone 1902), (syn. *Distoma pristiophori* Johnstone 1902).

Host: *Raja clavata* L.; locality: east coast of Iceland, 11° 14′ W., 65° 58′ N.; depth: 65 fathoms; incidence: 19 August 1948, one specimen; habitat: stomach.

The genus *Otodistomum* was proposed by Stafford (1904) for *Distoma veliporum* Creplin, 1837, which he obtained from the stomach of *Raja stabuliformis*. Odhner (1911) distinguished two species of the genus, namely, *Otodistomum veliporum* (Creplin) and *O. cestoides* (Beneden). The close morphological resemblance between these two species has led to much controversy as to whether or not they are synonymous. Manter (1926) considered that the Pacific forms belong to the species *veliporum* and the Atlantic forms to the species *cestoides*. Dollfus (1937) agreed that two species could be distinguished on the grounds of their geographical distribution and the species of host fish which they inhabit. The North Atlantic forms north of 50° N. for Europe and 40° N. for America and the North Pacific forms were placed by Dollfus in the species. *O. cestoides*, the hosts being *Chlamydoselachus* and *Raja*. This species he subdivided into two subspecies, *Otodistomum cestoides cestoides* from *Chlamydoselachus* and *Raja* in the North Atlantic and North Pacific and *O. cestoides pacificum* from *Raja* in the North Pacific. Forms found in the Atlantic of southern Europe, North Africa and Mediterranean, were placed by Dollfus in the species *Otodistomum veliporum*, the hosts being Squaliformes and Torpediniformes. He recognized three subspecies, namely, *O. veliporum veliporum*, *O. v. leptotheca* and *O. v. pachytheca*. Doll. ıs also considered *Distoma pristiophori* Johnstone, 1902, as belonging to the genus *Otodistomum*, so that, according to him, there are six forms belonging to the genus. If this arrangement were to be accepted, the present specimen should be identified as *O. cestoides cestoides*. The distinction between the above-mentioned forms is based on the size of the egg and the thickness of the egg-shell. Other features at one time used to differentiate *O. cestoides* and *O. veliporum* were the structure of the genital atrium and genital papilla, the arrangement of the vitellaria and the ratio of the suckers. According to Manter (1926), however, the differences between these structures are not constant in the two species and are therefore useless for specific diagnosis. Muhlschlag (1914) has pointed out that *O. cestoides* and *O. veliporum* are even more similar than was at one time thought, the only distinction between them being in the size of the egg and thickness of the shell. He adds that although the discrepancy in egg-shell thickness appears to be a constant feature it hardly seems to be in itself a specific diagnosis. Dawes (1946) has expressed doubt concerning the reliability of the size of the egg and thickness of the shell as diagnostic features, and van Cleave & Vaughn (1941) found an overlap in the size of the egg in *O. cestoides cestoides* and *O. c. pacificum*; therefore, these two subspecies relying on this character become invalid. Similarly, Miller (1941) found an overlap in the egg size in the case of *O. veliporum* and *Xenodistomum melanocystis* Stafford, 1904. Dawes is of the opinion, therefore, that if the egg size does not prove to be a diagnostic feature in two cases it ceases to be a reliable feature on which to base a species or subspecies. He therefore places all the forms in the one species, *Otodistomum veliporum*, this name taking precedence over *O. cestoides*. Heller (1949) agrees that Dawes's conclusion seems to be logical but she identifies specimens from *Raja scabrata* off the Canadian coast as *Otodistomum cestoides* until the problem is finally settled.

In the present instance Dawes's suggestion has been accepted and the specimen named *O. veliporum*. The genus is very widely distributed, occurring in about thirty-five different species of host fishes in various parts of the world. It seems reasonable to expect some minor morphological variations within such a wide variety of hosts scattered over such a wide area.

The species has not been recorded previously from Icelandic waters. It is reported by Nicoll (1915) as occurring off the east coast of Britain in *Raja batis* L., from Aberdeen and *R. radiata* Donovan from the Northumberland coast. The larval stage is recorded by the same writer encysted in the stomach wall of *Glyptocephalus cynoglossus* (L.) at Aberdeen. Manter (1926) records *Otodistomum cestoides* from *Raja binoculata* in Alaska and Friday Harbour, Washington. He also stated that *Otodistomum veliporum* is one of the common fish parasites in the region of Mount Desert Island and that Linton has found it occasionally at Wood's Hole. *O. pristiophori* was first recorded by Johnston in 1903 from the coelome of *Pristiophorus cirratus* at Sydney, and has been found by Woolcock (1935) in the same host species from Port Philip Bay, Victoria. It has been considered by Odhner (1911) and Manter (1934) as being synonymous with *Otodistomum veliporum*.

The diagnostic features of *O. veliporum* are as follows. Elongated and only slightly flattened; up to 80 mm. long; cuticle smooth and slightly wrinkled; ventral sucker 2·4 mm. in diameter, twice as large as the oral sucker and close behind it; pre-pharynx absent, pharynx well developed, caeca long, slightly sinuous and extending almost to posterior extremity; excretory bladder Y-shaped with long median stem and lateral branches extending to the pharynx; genital pore near pharynx and slightly to the left of the middle line; genital atrium large, cirrus-sac rounded, vesicula seminalis small and club-shaped; testes oval one behind the other near the middle of the body; ovary in front of and a little to the right of the anterior testis; receptaculum seminis absent; vitellaria extending from behind ventral sucker to the last fifth of body, approaching the middle line behind the testes; uterus with ascending limb only and folded transversely between the ovary and ventral sucker; eggs broadly oval, about 0·06 mm. long.

(4) **Derogenes varicus** (Müll., 1784)

Synonymy: *Fasciola varica* Müll. 1784 and Rud., 1802; *Distoma varicum* Zed. of Rud., 1809; *Distomum dendriticum* Creplin, 1829 in part; *Derogenes varicum* (Müll.) of Olsson 1868 and Levinsen, 1881 nec. Monticelli, 1890; *D. minor* Looss, 1901; *D. plenus* Stafford, 1904; *D. fuhrmanni* Mola, 1912; *D. crassus* Manter, 1934.

Hosts ...	*Platichthys flessus* (L.)	*Brosme brosme* (Müll.)
Locality	10° 54′W., 65° 55′N.	15° 15′W., 63° 47′N.
Depth	160 fathoms	160 fathoms
Incidence	20 August 1948, four specimens	21 August 1948, ten specimens
Habitat	Stomach	Stomach

According to Lebour (1908), Johnstone (1907), Nicoll (1914, 1915), and Baylis & Jones (1933), *Derogenes varicus* is universally distributed around the coasts of Britain, having been recorded from fishes landed at Aberdeen, Galway, St Andrews, Millport, Liverpool and Plymouth. It is the commonest fish trematode occurring in a wide variety of hosts. The hosts named for British waters are the following: *Mullus surmuletus* L., *Pagellus centrodontus* (De la Roche), *Trachurus trachurus* (L.), *Caprox aper* (L.), *Zeus faber* L., *Trachinus draco* L., *T. vipera* Cuvier & Valenciennes, *Lophius piscatorius* L., *Cottus scorpius* L., *C. bubalis* Euphrasen, *Agonus cataphractus* (L.), *Callionymus lyra* L., *Trigla cuculus* L., *Blennius ocellaris* L., *Gadus callarias* L., *G. luscus* L., *G. merlangus* L., *G. minutus* L., *G. pollachius* L., *G. virens* L., *Merluccius merluccius* (L.), *Molva molva* (L.), *Brosme brosme* (Müll.), *Onos tricirratus* (Bloch), *Scophthalmus maximus* (L.), *S. rhombus* (L.), *Platichthys flesus* (L.), *Limanda limanda* (L.), *Microstomus kitti* (Walbaum), *Pleuronectes platessa* L., *Solea solea* (L.), *Hippoglossus hippoglossus* (L.), *Reinhardtius platessoides* (Fab.), *Salmo trutta* L., *S. salar* L., *Clupea sprattus* L. and *Conger conger* (L.).

Nicoll (1915) records it from five species of British fish but outside British waters, namely, *Sebastes marinus* (L.), *Labrus bergylta* L., *Clupea harengus* L., *Anguilla anguilla* (L.) and *Acipenser sturio* L.

Derogenes varicus has been reported by Olsson (1868) from thirteen host fishes in Scandinavian waters. With the exception of *Molva abyssorum* and *Coregonus oxyrhynchus* (L.), the hosts are all included in the previous list. It also occurs quite frequently in Canadian and American waters. Stafford (1904) found it in sixteen species of host fish in Canadian waters, namely, *Salmo salar* L., *Gadus callarias* L., *G. virens* L., *G. aeglefinus* L., *Clupea harengus* L., *Sebastes marinus* (L.), *Anguilla anguilla* (L.), *Cottus scorpius* L., *Lophius piscatorius* L., *Hippoglossus hippoglossus* (L.), *Reinhardtius hippoglossoides* (Walbaum), *Hippoglossoides platessoides* (Fab.), *Anarhichas lupus* L., *Osmerus mordax*, *Cryptacanthodes maculatus*, *Hemitripterus americanus*. Miller (1941) also recorded it from Canadian waters in eight host fishes, all except one, namely *Anguilla chrysupa*, being included in the above list. Heller (1949) found it in three host species in the Baie de Chaleur region.

Manter (1926) recorded it from six host species from Maine, namely, *Gadus callarias* L., *Urophycis chuss* (Walbaum), *U. tenuis*, *Anarhichas lupus* L., *Hippoglossus hippoglossus* (L.) and *Myxocephalus octodecimspinosus*, and the same writer (1934) from five host fishes from the deep waters of the Gulf of Mexico, namely, *Merluccius* sp., *Urophycis regius* Walbaum, *Starches parmatus* Goode, *Helocolenus dactylopterus* (De la Roche), and *Scorpaena cristulata* Goode & Bean. Dawes (1946) mentions that it occurs near Banyuls and off the coast of the Galapagos Islands.

It can be seen, therefore, that the species is widely distributed geographically, and occurs in a very large number of host fishes. The two host records in the present instance are not new, but *Derogenes varicus* has not been found previously off the coast of Iceland.

There are several adequate descriptions of this trematode. The diagnostic features are as follows: 1·5–7·0 mm. long with spindle-shaped body narrowing anteriorly; cuticle unarmed; ventral sucker 0·33–0·60 mm. in diameter, oral sucker 0·19–0·30 mm. ventral sucker situated far back; no prepharynx, short oesophagus, caeca extending to posterior extremity; excretory vesicle Y-shaped; genital pore far in front of the ventral sucker and close behind the bifurcation of the intestine; hermaphrodite duct short; pars prostatica long, surrounded by large

conspicuous cells free in the parenchyma; vesicula seminalis prominent; testes ovoid, somewhat diagonally placed close behind the ventral sucker; ovary spherical, behind the testes; vitellaria two oval masses a short distance in front of the posterior end of the body; uterus with much folded descending and ascending limbs; eggs 0·050–0·026 × 0·025–0·034 mm.

Measurements, in mm., taken from specimens in the present investigation are as follows: length 1·50–3·85; breadth 0·47–1·00; oral sucker 1·19–0·30; ventral sucker 0·33–0·60; eggs 0·05–0·06 × 0·031–0·035.

(5) **Gonocerca crassa** Manter, 1934

Host: *Molva byrkelange* Walbaum; locality: east coast of Iceland, 15° 15′ W., 63° 47′ N.; depth: 160 fathoms; incidence: 21 August 1948, one specimen; habitat: stomach.

Gonocerca crassa was first described by Manter (1934) from thirteen host fishes off Tortugas, Florida, namely, *Ancylopsetta delecta* (Goode & Bean), *Brotula barbata* (Bloch & Schenider), *Coelorhynchus carminatus* (Goode), *Lophius piscatorius* L., *Merluccius* sp., *Paralichthys oblongus* (Mitchell), *Paralichthys* sp., *Setarches parmatus* Goode, *Synodus intermedius* (Agassiz), *Saureda* sp., *Synodontia* sp., *Urophycis cirratus* (Goode & Bean), *U. regius* (Walbaum).

In the present instance one specimen was obtained from the stomach of *Molva byrkelange* off the coast of Iceland. This is a new host and locality record. As far as the writer is aware, no further record of the parasite exists. As only one specimen was available no further description of the species was possible. It agrees, however, with the description given by Manter.

The diagnostic features of *Gonocerca crassa* are as follows: body cylindrical, fairly thick; 2·24–3·57 mm. long, 0·798–0·420 mm. broad; ventral sucker large and situated in the posterior part of the body, a little less than twice the diameter of the oral sucker; oral sucker slightly wider than long, preoral lobe well developed; prepharynx absent, oesophagus short, caeca broad and extending past the testes to the posterior extremity; genital pore median and close behind the oral sucker; genital atrium short and wide, genital cone capable of extrusion, genital sinus short, prostate gland region fairly short, vesicula seminalis small tubular undivided and sometimes curved, rarely extending posteriorly more than half way between the suckers; testes large and globular situated almost side by side near the posterior end of the body; ovary median immediately behind the ventral sucker; vitellaria compact, large, one on each side partly posterior to the ovary; receptaculum seminis and Laurer's canal absent; uterus entirely pre-ovarian almost wholly preacetabular with short lateral coils chiefly intercaecal; egg large and yellow, 0·041–0·052 × 0·020–0·022 mm.

The measurements, in mm., of the present specimen agree very closely with those given by Manter. They are as follows: length 3·53; breadth 1·00; oral sucker 0·41; ventral sucker 0·77; pharynx 0·135; eggs 0·040 × 0·019.

(6) **Hemiurus communis** Odhner, 1905

Synonymy: *Distoma appendiculatum* of Olsson, 1868 and Juil, 1889, in part, nec. Rud., 1802; *D. appendiculatum* of Johnstone, 1907.

Host: *Sebastes marinus* (L.); locality: east coast of Iceland, 11° 40′ W., 66° 50′ N.; depth: 58 fathoms; incidence: 18 August 1948, one specimen; habitat: stomach.

According to Nicoll (1914) *Hemiurus communis* is, with the exception of *Derogenes varicus*, the most widely distributed of all British fish parasites. The hosts recorded around the British coasts by Lebour (1908, 1935), Nicoll (1914, 1915), Baylis & Jones (1933) and Dawes (1946) are numerous, including *Conger conger* (L.), *Gadus callarias* L., *G. aeglifinus* L., *G. luscus* L., *G. minutus* L., *G. merlangus* L., *G. virens* L., *G. pollachius* L., *Molva molva* (L.), *Brosme brosme* (Müll.), *Ammodytes lanceolatus* Lesauvage, *A. tobianus* L., *Trigla lucerna* L., *T. cuculus* L., *T. gurnardus* L., *T. lineata* Gmelin, *Cottus scorpius* L., *C. bubalis* Euphrasen, *Zeugopterus punctatus* (Bloch), *Hippoglossus hippoglossus* (L.), *Limanda limanda* (L.), *Lophius piscatorius* L., *Reinhardtius platessoides* (Fab.), *Lepadogaster gouani* Lacepède, *Pholis gunnellus* (L.), *Gobius paganellus* Gmelin, *Anguilla anguilla* (L.), *Capros aper* (L.), *Trachurus trachurus* (L.), *Trachinus draco* L., *Pagellus centrodontus* (De la Roche), *Entelurus aequoreus* (L.), *Platichthys flesus* (L.) and *Pleuronectes platessa* L.

The localities from which these fishes have been obtained include Plymouth, New Brighton, Piel (Barrow in Furness), Galway, St Andrews, Millport, Aberdeen and Cullercoats. According to Odhner the parasite is also common in many Scandinavian fishes.

Nicoll (1914) stated that although this trematode is so widely distributed, it shows a distinct preference for Gadoid fishes. Frequently only one specimen occurs in each fish, as in the present instance, but occasionally there may be more. Lebour (1908) found two to three in specimens of *Gadus merlangus* off the Northumberland coast, and Baylis & Jones (1933) twelve in one specimen of *Cottus bubalis* at Plymouth.

Hemiurus communis has not been recorded previously from *Sebastes marinus*, nor from Icelandic waters.

The diagnostic features of *Hemiurus communis*

are as follows: 2·0–2·8 mm. long with ecsoma extended, 0·40–0·50 mm. broad narrowing slightly anteriorly; cuticle annulated ventrally as far as end of soma, striae fine on the ecsoma; ventral sucker 0·30 mm. in diameter and almost twice the diameter of the oral sucker; prepharynx absent, pharynx and short oesophagus present, caeca extending for a short distance into the ecsoma; genital aperture beside or in front of the pharynx to the left of the middle line; hermaphrodite duct fairly thick being about equal in length to the diameter of the ventral sucker; pars prostatica long and winding with large gland cells; vesicula seminalis bipartite; testes one behind the other close behind the ventral sucker, anterior testis smaller than the posterior; ovary rounded situated in the last third of the soma; receptaculum seminis present; vitellaria irregularly rounded lying a short distance behind the ovary; uterus with descending and ascending limbs, extends into ecsoma; eggs 0·019–0·026 × 0·010–0·012 mm.

H. communis has been adequately described by Olsson (1868) and Lebour (1908).

The measurements, in mm., of the single specimen found in the present study are as follows: length 2·40; breadth 0·30; oral sucker 0·18; ventral sucker 0·35; pharynx 0·10; eggs 0·025 × 0·013.

(7) **Lepidophyllum steenstrupi** Odhner, 1902

Host: *Anarhichas lupus* L.; locality: east coast of Iceland, 12° 33′ W., 66° 27′ N.; depth: 65 fathoms; incidence: 17 August 1948, two specimens; habitat: urinary bladder.

This species has not been recorded around the coasts of the British Isles but has been found in European and Canadian waters. *Lepidophyllum steenstrupi* was first described by Odhner (1902) from the urinary bladder of *Anarhichas lupus* L. Stafford (1904) recorded it from *A. lupus* and *Zoarces anguillaris* Peck in Canada, and Miller (1941), revising Stafford's collection, stated that there were five specimens, only one of which contained eggs in the uterus. Dawes (1946) stated that the species occurs in *Anarhichas lupus* in Norway, *A. pantherinus* in Iceland and *Zoarces anguillaris* in Canada. The writer has not been able to trace the original record of the species from Icelandic waters. Two specimens only were found in the present instance. Had more been available a further and more complete study of the anatomy could have been made. Both specimens have been mounted permanently so none is available for sectioning.

The diagnostic features of *Lepidophyllum steenstrupi* are as follows: 2·1–2·4 mm. long, 1·1–1·4 mm. broad; posterior end rounded, being widest at the level of the testes and tapering gradually to the anterior end; cuticle covered with transverse rows of fine spines; oral sucker almost terminal 0·18 mm. in diameter, ventral sucker slightly smaller and a little in front of the middle of the body; prepharynx short, pharynx well developed, oesophagus twice as long as pharynx, caeca extending to about middle of body; excretory vesicle tube-shaped and short, excretory pore dorsal; genital pore dorsal on left side near level of forking of intestine; cirrus-sac extends obliquely backwards to the middle line and anterior margin of the ventral sucker; testes lobed, one on each side behind caeca; ovary rounded situated a short distance behind ventral sucker and a little to the left of the middle line; receptaculum seminis behind ovary, in middle line; vitellaria consists of eight to eleven small follicles on each side situated on the inner side of and slightly anterior to the testes; uterus with descending and ascending limbs; eggs 0·043 × 0·021 mm. with thick shells.

The measurements, in mm., of the two specimens in the present investigation are given in Table 4.

Table 4

	(1)	(2)
Length	2·00	1·41
Breadth	1·74	1·45
Oral sucker	0·19	0·17
Ventral sucker	0·18	0·15
Pharynx	0·14	0·13
Eggs	0·043 × 0·021	0·043 × 0·020

Both specimens were mature. Specimen 1 contained a number of eggs in the uterus and in specimen 2 only two eggs were present.

(8) **Steganoderma pycnorganum** n.sp.

Host: *Anarhichas minor* Olafsen; locality: east coast of Iceland, 12° 33′ W., 66° 27′ N.; depth: 65 fathoms; habitat: bile duct.

The body is stout, oval in outline, broadest about the middle and tapering anteriorly and posteriorly to rounded extremities (Fig. 1). It measures 2·44–2·82 mm. in length with a maximum breadth of 1·41–1·52 mm. The cuticle is armed over the whole surface with transverse rows of spines. The oral sucker is subterminal and measures 0·358 × 0·336 mm., the breadth being slightly greater than the length. The ventral sucker measures 0·624 × 0·622 mm. and is situated at about the middle of the body length or slightly in front of it.

The mouth leads into a short prepharynx followed by a pharynx measuring 0·143 mm. in diameter. The oesophagus, 0·20 mm. long, is nearly twice the length of the pharynx; it bifurcates a little way in front of the ventral sucker into two short sac-like intestinal limbs. These diverge from one another and terminate on either side opposite the middle of the ventral sucker and some little distance in front of the testes.

The excretory vesicle is Y-shaped. The main stem is fairly long, extending from the terminal pore forwards to the level of the testes where it bifurcates. It was not possible to follow the excretory vessels farther forwards. The terminal stem is surrounded by small gland cells which are best developed near the excretory pore.

join one another dorsally to the ovary forming the vas deferens. The vas deferens continues forwards to enter the cirrus-sac (Figs. 1 and 2). The cirrus-sac is very large, measuring 0·98 mm. in length; it is broadest proximally where it measures 0·26 mm. in diameter and narrows slightly towards the genital atrium. It extends in a curve from the genital

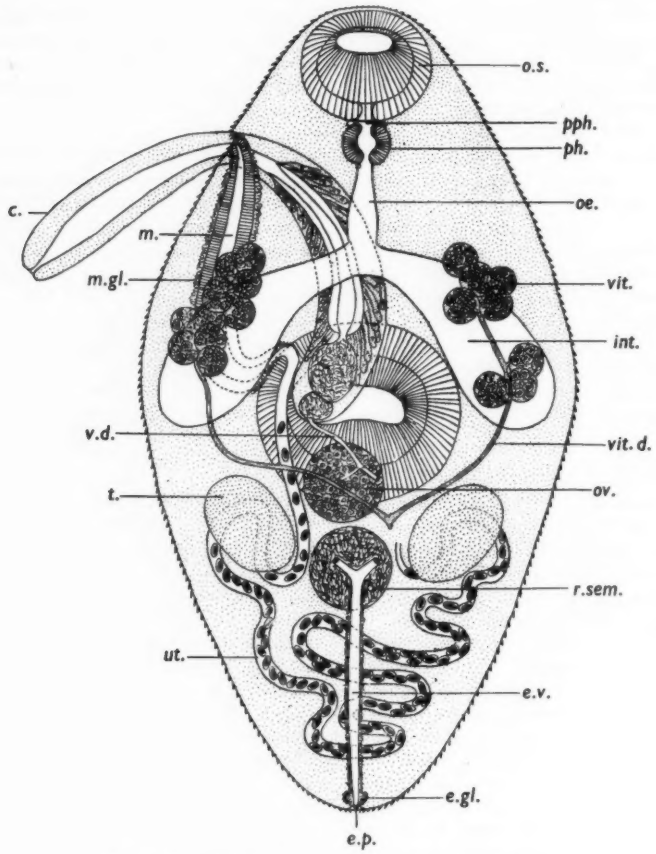

Fig. 1. *Steganoderma pycnorganum* n.sp., dorsal view.

The genital atrium lies on the left lateral margin in the neighbourhood of the pharynx. The female duct opens into the genital atrium dorsally to the male duct (Figs. 1 and 2).

The testes are fairly large oval bodies measuring 0·27–0·38 × 0·23–0·33 mm. and are symmetrically arranged close behind the ventral sucker, the longitudinal axes being oblique (Fig. 1). The point of origin of the vasa efferentia could not be seen in whole mounts but they could be traced from near the testes as they pass towards the middle line to

atrium towards the middle line ventrally to the gut and overlaps the anterior half of the ventral sucker (Fig. 1). Within the cirrus-sac the vas deferens enlarges to form a bipartite vesicula seminalis (Fig. 2), the distal portion being the larger, measuring 0·25–0·27 × 0·12–0·13 mm. and the proximal smaller, measuring about 0·076 × 0·054 mm. The two portions are connected by a short narrow tubular region which is usually folded on itself. The vesicula seminalis continues into the pars prostatica, being separated from it by a constriction. The pars pro-

statica is about half the length of the cirrus-sac and into it open the prostatic gland cells which occupy a considerable part of the cirrus-sac. The pars prostatica is followed by the ductus ejaculatorius. The evaginated cirrus is very long, measuring 0·870 × 0·146 mm. and is not armed with spines. The surface is, however, covered with small papillae

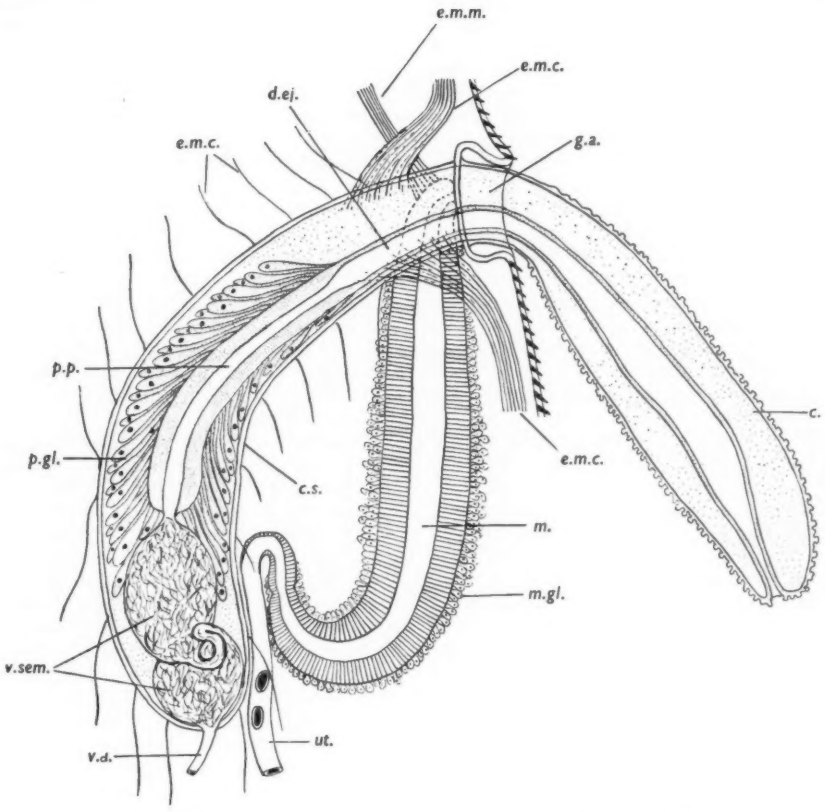

Fig. 2. *Steganoderma pycnorganum* n.sp., cirrus-sac metraterm, ventral view.

equally apparent in the invaginated and evaginated condition. The large and powerful nature of the cirrus-sac necessitates its being anchored by extrinsic muscles so that during evagination and invagination of the cirrus it is not torn away from the surrounding tissues. As far as can be seen in whole mounts these extrinsic muscles have their origin in the longitudinal muscles of the body wall. Near the genital atrium are two conspicuous extrinsic muscles one inserted into the wall of the cirrus-sac anteriorly and the other posteriorly (Fig. 2). The anterior bundle originates from the longitudinal muscles of the body wall laterally and appears as a group of fibres which turn inwards in front of the genital atrium and are inserted into the wall of the cirrus-sac a short distance before it reaches the genital atrium. Some of the fibres extend for a short distance on to the dorsal surface of the cirrus-sac and others on to the ventral surface. The posterior extrinsic muscles originate in the same way a short distance behind the cirrus-sac. This bundle of muscles runs forwards, turning inwards just behind the genital atrium, the fibres being inserted into the wall of the cirrus-sac, some on to the dorsal and some on the ventral side. This region of the cirrus-sac is therefore held firmly in position. The remainder of the cirrus-sac along its whole length is provided with fairly regularly arranged extrinsic muscle fibres not aggregated into bundles and originating from the longitudinal muscles of the body wall over a fairly wide area. The cirrus-sac as

a whole, therefore, is firmly anchored which allows for more efficient evagination and invagination of the cirrus.

The ovary is median and overlaps the posterior margin of the ventral sucker (Fig. 1). It is rounded in outline and measures 0·25 mm. in diameter. Behind the ovary in the middle line is the receptaculum seminis slightly larger than the ovary and measuring 0·27 mm. in diameter. The ovary and receptaculum seminis lie between the right and left testes, so that all these organs are somewhat crowded together near the posterior margin of the ventral sucker. The oviduct and neighbouring ducts could not be seen in whole mounts. The early part of the uterus passes backwards on the inner side of the right testis and describes a few coils in the posterior region of the body behind the genitalia. Some of the coils extend forwards on either side, overlapping the testes ventrally. The ascending limb of the uterus passes forwards on the inner side of the left testis, runs dorsally to the left margin of the ventral sucker, and becomes continuous with the metraterm. The metraterm is a conspicuous structure about equal in length to the evaginated cirrus (Figs. 1 and 2). It measures 0·80 mm. in length and 0·11 mm. in breadth. It extends from the genital atrium in a curve to the anterior margin of the ventral sucker, being a little shorter than the cirrus-sac. The wall of the metraterm is thick (Fig. 2), measuring 0·031–0·043 mm. It is surrounded along its length by unicellular glands and opens into the genital atrium dorsally to the cirrus. One bundle of extrinsic muscles has been found associated with the metraterm. The fibres which comprise it originate from the longitudinal muscles of the body wall. They pass backwards and outwards to be inserted into the anterior portion of the wall of the metraterm immediately before it opens into the genital atrium (Fig. 2). The uterus is packed with eggs which have brown shells and measure 0·031–0·038 × 0·019–0·020 mm.

The yolk glands consist of a few large follicles on either side (Fig. 1). The number on both sides is not constant, there being more on the left than on the right. An average for the six specimens obtained was nine on the right and eleven on the left, but they can vary, for example, from eight on the right and twelve on the left or nine on the right and ten on the left or eleven on the right and twelve on the left. They are situated ventrally to the intestinal limbs in the anterior part of the body, mainly in front of the ventral sucker. The individual follicles vary slightly in size, those of the right side where there are fewer are usually slightly larger than those of the left side. Measurements of some of the follicles are as follows: 0·093 × 0·062 mm., 0·120 × 0·100 mm., 0·135 × 0·104 mm. and 0·154 × 0·104 mm. Small ducts from the individual follicles unite to form the lateral yolk duct on either side. These pass backwards, turning towards the middle line and unite dorsally to and slightly to the right of the ovary to form the yolk reservoir. The median yolk duct which opens into the oviduct could not be traced.

There is no doubt that this species belongs to the subfamily Lecithostaphylinae of the family Zoogonidae. It has been placed in the genus *Steganoderma*, though in some respects it resembles three other genera included in the Lecithostaphylinae, namely, *Lecithostaphylus* Odhner, 1911, *Deretrema* Linton, 1910, and *Brachyenteron* Manter, 1934. It is quite distinct from *Lepidophyllum* Odhner, 1902, and *Paralepidophyllum* Yamaguti, 1934. Only a limited number of species has been described in this subfamily, so it is possible that as more species are discovered slight alterations will be necessary in the generic diagnoses to allow of their inclusion. The genus *Steganoderma* was first described by Stafford (1904) with *S. formosum* from the intestine and caeca of *Hippoglossus hippoglossus* in Canada as the type species. Yamaguti (1934) described two other species, namely, *Steganoderma fellis* from the gall bladder of *Sillago sihama* and *Steganoderma sebastodis* from the gall bladder of *Sebastodes inerma* at Tarumi. *Nordosttrema messjatzevi* Issaitschikow (1928) is considered by Yamaguti to be a synonym of *Steganoderma*.

In the position of the ventral sucker the present species resembles *Steganoderma*, *Lecithostaphylus* and *Brachyenteron* where it is described as being in front of the middle of the body, though in the present instance it is only very slightly so. In most species belonging to the above-mentioned genera both suckers are almost equal in size. In the present case the ventral sucker is nearly twice the diameter of the oral sucker as in *Steganoderma sebastodis* Yamaguti and in the genus *Deretrema*. The pharynx is larger than is usually described for *Steganoderma* and *Brachyenteron* and the oesophagus is long, which latter feature separates it from *Lecithostaphylus*. In the extent of the caeca the present form lies between *Brachyenteron* and the other three genera mentioned. The genital pore on the left lateral margin opposite the pharynx is further forwards than in *Steganoderma*, *Deretrema* or *Brachyenteron*, and more nearly resembles in position that of *Lecithostaphylus*. The large cirrus-sac is a common feature, but the elongated cirrus is not found in *Lecithostaphylus*, the vesicula seminalis is bipartite as in *Steganoderma* and *Brachyenteron* and the testes are close to the posterior border of the ventral sucker as in *Brachyenteron* and *Deretrema*. The yolk follicles are few in number as in all these genera, there being slightly more on the left than on the right. In almost all the forms described in the subfamily Lecithostaphylinae the yolk follicles are situated between the ventral sucker and the testes, not extending beyond the anterior margin of the ventral sucker. In the

present instance, however, they are mainly pre-acetabular, a feature which it has in common with *Steganoderma fellis* and *S. sebastodis*. These two latter inhabit the gall bladder of their hosts as does the present species.

On balance, therefore, the present species has more features in common with *Steganoderma* than with the other genera, and so has been placed there under the name of *S. pycnorganum*.

Specific diagnoses. Fairly stout, 2·44–2·82 mm. long with a maximum breadth of 1·41–1·52 mm.; cuticle covered with spines; ventral sucker at or slightly in front of middle of body, not quite twice the diameter of the oral sucker; prepharynx short, pharynx well developed, oesophagus long, intestinal caeca short and sac-like terminating near the level of the middle of the ventral sucker; genital pore on left lateral margin near pharynx; cirrus-sac large overlapping anterior margin of ventral sucker; vesicula seminalis bipartite, pars prostatica occupies about half cirrus-sac; cirrus long covered with papillae; metraterm long and muscular; testes symmetrical close behind ventral sucker; ovary median overlapping ventral sucker; receptaculum seminis behind ovary; yolk follicles large, 8–11 on right and 10–12 on left, mainly pre-acetabular; eggs 0·031–0·038 × 0·019–0·020 mm.

Species of the subfamily Lecithostaphylinae have not appeared in Britain, but some of them occur in European waters. No species of *Steganoderma* have been recorded for Europe, but occur in fishes off the coast of Canada, Florida and Japan. The only member of the subfamily previously recorded from fishes in Icelandic waters is *Lepidophyllum steenstrupi* which has also been found during the present investigation.

DISCUSSION

Eight species of digenetic trematodes have been obtained from fishes trawled off the coast of Iceland. With the exception of *Derogenes varicus* each has been recorded from only one species of host fish. Six of the seven known species have been recorded for the first time from this locality, the exception being *Lepidophyllum steenstrupi*. There are two new host records, namely, *Hemiurus communis* from *Sebastes marinus* and *Gonocerca crassa* from *Molva byrkelange*. One new species, *Steganoderma pycnorganum*, has been described from *Anarhichas minor*.

Fishes examined in the present investigation were obtained from depths of 58–160 fathoms and may be regarded as deep-water fishes. Stafford (1904) considers deep-water fishes to be those caught in the trawl from a depth of 40 fathoms downwards. Of the many records of parasites of fishes few give the depths from which the fishes were taken. The greatest depth at which an adult trematode has yet been found is 1090 fathoms, the trematode being *Distomum halosauri* recorded by Bell in 1887 from *Halosaurus macrocher* collected by the *Challenger* expedition. According to Stafford von Linstow, in his report on the *Challenger* expedition, does not record any trematodes from deep-water fishes. He assumes their absence to be due to the fact that the larvae are too delicate to withstand the pressure of a deep-sea life. Stafford, however, found many fish trematodes in deep water, e.g. *Otodistomum* (?) from 300 to 390 fathoms, *Gonocerca phycidis* from 139 to 367 fathoms, *Derogenes varicus* from 190 to 367 fathoms, *Gonocerca crassa* from 40 to 300 fathoms, and *Hemiperina nicolli* from 249 to 300 fathoms. In his investigations into the bathymetric distribution of trematodes he found, altogether, forty-nine species in deep water. Trematodes with only one definitive host are naturally limited by host specificity rather than depth, e.g. *Lomophorus monolenei*, with only one host, occurs only at depths of 100–150 fathoms. *Derogenes varicus*, on the other hand, which is the most widely distributed and least specific fish trematode, occurs at depths of 160–367 fathoms. *Gonocerca crassa*, too, has a wide host range and consequently depth range which extends from 40 to 300 fathoms. Stafford found that the trematode fauna from deep-water fish of Tortugas more nearly resembled the fauna from cooler shallow waters in more northerly regions such as the coastal waters of Britain and Norway than the fauna of the warm surface waters of Tortugas. He suggested, therefore, that temperature is an important factor in the distribution or marine fish trematodes. It is doubtful whether temperature variations could have a direct effect on the distribution of adult trematodes within the fish hosts. It seems more likely that the temperature factor works indirectly by determining the distribution of the fish host and of any intermediate hosts and by its effect on the survival of free-living larval stages of the parasites.

In the present investigation the data available are insufficient to draw any definite conclusions. Two of the trematodes recorded by Stafford for the deep waters of Tortugas occur off the coast of Iceland, namely, *Derogenes varicus* and *Gonocerca crassa*. Both of these occur at a depth of 160 fathoms, the greatest depth from which fish were obtained in the present instance. *Derogenes varicus* in Tortugas occurs at a depth of 190–367 fathoms, and so seems to be characteristic of deep water in both localities. Two other fishes obtained off the coast of Iceland, namely, *Anarhichas lupus* and *Sebastes marinus*, have been recorded as harbouring this trematode in British coastal waters but no specimens were found in them at depths of 58 and 65 fathoms respectively in Icelandic waters. *Gonocerca crassa* has the wide range of distribution of from 40 to 300 fathoms in Tortugas. Off the coast of Iceland it was found at 160 fathoms, which comes within this range. None

of the hosts recorded for it in Tortugas has been obtained in Icelandic waters. Stafford found the deepest water trematodes to belong to the family Fellodistomatidae, some occurring at a depth of 582 fathoms. In the present instance the only member of the family, *Fellodistomum fellis*, occurred in much shallower water at a depth of 65 fathoms. It seems that considerably more work will be necessary on the trematode parasites of fishes and their life histories before any definite conclusions can be made about their bathymetric distribution.

Trematodes of fishes are not as a rule numerous within a single host. In the present case only one parasite has sometimes been found, e.g. *Lebouria idonea* from *Anarhichas lupus*, *Otodistomum veliporum* from *Raja clavata*, *Hemiurus communis* from *Sebastes marinus*, and *Gonocerca crassa* from *Molva byrkelange*. The maximum number was in the case of *Derogenes varicus*, where ten specimens were recovered from the stomach of a single specimen of *Brosme brosme*. The next most abundant was *Steganoderma pycnorganum* n.sp., where six specimens were obtained from the gall bladder of *Anarhichas minor*. The degree of infestation of the final host must of course be correlated in part with the distribution and availability of the infected intermediate hosts.

SUMMARY

1. Eight species of digenetic trematodes, including one new species, have been recorded from six species of host fishes caught in the trawl off the east coast of Iceland.

2. Seven species are recorded for the first time from this locality and there are two new host records among the seven known species.

3. Details of the exact locality and depth from which the fishes were taken are given, together with a brief account of each known trematode and a more extensive account of the new species, *Steganoderma pycnorganum*.

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Key to lettering of figures

c.	cirrus	*g.a.*	genital atrium	*ph.*	pharynx
c.s.	cirrus-sac	*int.*	intestinal caecum	*pph.*	prepharynx
d.ej.	ductus ejaculatorius	*m.*	metraterm	*r.sem.*	receptaculum seminis
e.gl.	glands surrounding excretory vesicle	*m.gl.*	glands surrounding metraterm	*t.*	testis
		o.s.	oral sucker	*ut.*	uterus
e.m.c.	extrinsic muscles of cirrus-sac	*oe.*	oesophagus	*v.d.*	vas deferens
e.m.m.	extrinsic muscle of metraterm	*ov.*	ovary	*v.sem.*	vesicula seminalis
e.p.	excretory pore	*p.gl.*	prostatic gland cells	*vit.*	vitellaria
e.v.	excretory vesicle	*p.p.*	pars prostatica	*vit.d.*	vitelline duct

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A RECORD OF SOME PARASITIC WORMS FROM WHALES IN THE ROSS SEA AREA

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The material examined in the present investigation was given to the writer by Mr W. Ross Cockrill, F.R.C.V.S., Veterinary Officer, Ministry of Agriculture and Fisheries, Carmarthen. The specimens were collected by Mr Ross Cockrill on the whale factory ship *Balaena* when he accompanied the Antarctic Whaling Expedition in the winter of 1951–2 for the purpose of making investigations into the pathology of whales. The whales were caught in the Ross Sea area roughly between 67° 51′ S. and 71° 13′ S., and the parasitic worms recovered from them include cestodes, nematodes and Acanthocephala. The worms were fixed at the time of collecting, the cestodes in 5% formalin and the nematodes and Acanthocephala in hot 70% alcohol. When only one or a few specimens were found they were all fixed, but if the infestation was heavy a sample was collected. In addition, portions of infested kidney and lesions from other parts of the body thought to be associated with parasitic infestation were included in the collection. The writer is greatly indebted to Mr Ross Cockrill for the gift of this material.

Parasitic worms were collected from four species of whales, namely, *Balaenoptera musculus* L. (Blue whale, Sulphur-bottom, Sibbald's rorqual), *B. physalus* L. (Fin whale, Finback, Finner, Common rorqual), *Megaptera nodosa* Bonn. (Humpback) and *Physeter catodon* L. (Sperm whale, Cachalot). From these hosts five species of cestodes, two of nematodes and two Acanthocephala were recovered. One of the cestodes, belonging to the genus *Tetrabothrius* from *Balaenoptera musculus*, is unidentified and may be new. There are two new host records, namely, two species of cestodes from *Physeter catodon*. Many of the helminth parasites of whales are imperfectly known and a redescription of some of them, particularly the cestodes, would be a useful contribution to their study. Dr Markowsky, of the Natural History Museum, has recently commenced a revision of the *Discovery* collection of cestodes from Cetacea, so no description of them will be given here. The writer is much indebted to Dr Markowsky for allowing her to examine some of his preparations and to verify some of the identifications of the present specimens. The unidentified species of *Tetrabothrius* is included in the *Discovery* material and will doubtless be described by Dr Markowsky.

The present paper, therefore, is a record of the helminths collected from whales during the 1951–2 Antarctic Whaling Expedition. A list of the parasites arranged in sytematic order is given, together with a few notes on each species, and this is followed by a list of hosts with the parasites recorded from each. For the purposes of identification whole mounts and sections have been made of the cestodes, the nematodes and Acanthocephala have been cleared in lactophenol or creosote and, where necessary, dissected.

The appendix at the end of the paper includes notes on some ectoparasites of whales collected during the expedition.

CLASSIFIED LIST OF PARASITES

CESTODA

Order **Cyclophyllidea**

Family Tetrabothriidae

1. *Priapocephalus grandis* Nybelin, 1922

Host. *Physeter catodon* L. (Sperm whale, Cachalot).

Occurrence. One specimen from the small intestine of a 52 ft. Sperm whale; Ross Sea area; 9 January 1952.

Priapocephalus grandis was first named by Nybelin (1922) but had been reported earlier on two occasions, namely, by Hamilton (1916) and Chapin (1921) from the Blue whale *Balaenoptera musculus*. The species was not fully described or named by either of these writers, but Chapin in his address to the Helminthological Society of Washington mentioned a remarkable hitherto unknown cestode from a Blue whale caught off Cape Point, South Africa, having a peculiar cylindrical scolex which could bury itself in the intestinal mucosa, 'riveting' the parasite to its host. Baylis (1926) received from H.E. the Governor of the Falkland Islands some

parasites of whales from Deception Island, South Shetlands, among which similar cestodes were included. In the meantime, however, Nybelin had described and named the species. Baer (1932) recorded *Priapocephalus grandis* from *Balaenoptera borealis* Lesson (Sei whale, Pollack whale, Rudolphi's whale) and from '*Balaena antarctica*' Gray (? *B. australis*, the Southern right whale). Baylis (1932) included it in his list of parasitic worms from Cetacea, recording it from *Balaenoptera musculus*, *B. borealis* and '*Balaena antarctica*'. Joyeux & Baer (1936) stated that it occurs in *Balaenoptera borealis* and *B. physalus*.

Priapocephalus grandis has not previously been recorded from the Sperm whale. It may be an unusual host, as only one specimen was found on this occasion. It measured 400 mm. in length with a maximum breadth of 8 mm. The scolex, 10 mm. long and 5·5 mm. broad, is the most striking feature of the worm. It is bulb-like in shape, devoid of suckers and surrounded at the base by a collar-like expansion. The scolex is embedded in the intestinal mucosa of the host up to the level of the collar, which presumably prevents it from penetrating further.

2. *Tetrabothrius affinis* (Lonnberg, 1891), Lonnberg, 1892

Synonyms. *Diplobothrium affine* Lonnberg, 1891; *Tetrabothrium* (*Diplobothrium*) *affine* Lonnberg, 1892.

Host. *Balaenoptera musculus* L. (Blue whale, Sibbald's rorqual, Sulphur-bottom).

Occurrence. Twenty specimens from the intestine of four Blue whales; Ross Sea area; 6–9 January 1952. The details of infestation are as follows:

Whale 1, 6 January: two specimens from an 82 ft. female Blue whale. The worms were found in the bile duct, but it is suggested by Mr Ross Cockrill that they passed into the bile duct from the intestine during the lemming process.

Whale 2, 7 January: eight specimens from the intestine of a 72 ft. female Blue whale; three have been fixed while still attached to the intestinal wall. The total infestation, according to Mr Ross Cockrill, did not exceed one hundred.

Whale 3, 17 January: six specimens from an 83 ft. male Blue whale. These were picked up on the plan after lemming, so the exact location is not known. The total infestation was apparently heavy.

Whale 4, 19 January: four specimens from the intestine of an 80 ft. male Blue whale. The total infestation was moderate.

Lonnberg (1892) obtained his material from *Balaenoptera borealis* taken off Finmark, Norway. He gave a detailed description of the species as far as the degree of maturity of his specimens would allow. He described the scolex as being quadrangular with rounded corners, the four large bothridia occupying each a quarter of the quadrant, their outer surfaces being spherical and their openings directed forwards. Baylis (1926) obtained specimens of the same worm from *B. musculus* from the South Shetlands and Cape Town, South Africa, and from *B. borealis* off Cape Point, South Africa. The species therefore occurs in both northern and southern waters. Baylis gave some further account of the anatomy and stated that 'the amount of variation possible in the shape of the scolex according to the state of contraction is surprisingly great'. The same has been found to be the case in the specimens examined in the present investigation. In the extended state when the scolex is relaxed the suckers hang down from the anterior part of the scolex, the 'auricular appendages' and 'epaulettes' being large and conspicuous. When contracted, however, and when attached to the host's intestine the posterior edges of the bothridia are drawn forwards so that all the suckers face anteriorly and the 'auricular appendages' and 'epaulettes', though still recognizable, become very much reduced in size. This condition was best seen in the specimens fixed while still attached to the intestinal wall of the host and removed after fixation. Baylis (1932) included the species in his list of parasites from Cetacea, and Baer (1932) recorded it from *B. borealis* and *B. musculus*. He confirmed Baylis's description after examining, as Baylis had done, Lonnberg's original material from Uppsala Museum. One of the features of *Tetrabothrius affinis* is the position of the yolk gland which lies ventrally to the ovary, a somewhat exceptional position in the Tetrabothriidae.

Of the twenty specimens examined in the present instance the largest measured 127 mm. with a maximum breadth of 5 mm., and the shortest 7 mm. in length with a breadth of 2 mm. Average measurements for the twenty were length 47 mm. and maximum breadth 3·6 mm. The scolex measures 4 mm. in breadth and 2 mm. in length. One curious feature seen in many specimens of *T. affinis* is a chain of very narrow squarish segments at the posterior end of the worm. In one specimen with a total length of 40 mm. the last 12 mm. consisted of such segments, the transition from the wide to the narrow region being a sudden one. The narrow segments contained reproductive organs, but the genital atrium in many cases, instead of opening on the lateral margin, was either mid-dorsal or mid-ventral.

3. *Tetrabothrius ruudi* Nybelin, 1928

Host. *Balaenoptera physalus* L. (Fin whale, Finback, Finner, Common rorqual).

OCCURRENCE. Four specimens from the small intestine of a male Fin whale; Ross Sea area; 22 January 1952.

Tetrabothrius ruudi was first described by Nybelin (1928), who obtained three specimens from a young male Fin whale. The account of the species is relatively brief, the main diagnostic features being the poorly developed 'auricular appendages' on the scolex, poorly developed muscles, 51–64 testes measuring 0·063 mm. in diameter, the armed cirrus, the ventral yolk gland and the extent of the gravid uterus which passes laterally beyond the excretory vessels.

Baer (1932) pointed out that *T. ruudi* resembles, in some respects, *T. affinis*. In both, the yolk gland is ventral to the ovary. In *T. affinis*, however, there are more than 100 testes in each segment and the worm itself is of stouter build. Baer has evolved a key for the identification of the four species of *Tetrabothrius* so far recorded from Cetacea, namely, *T. affinis* (Nybelin), *T. ruudi* Nybelin, *T. wilsoni* (Leiper & Atkinson) and *T. forsteri* (Krefft), based on the position of the yolk gland, the number of testes and the size of the scolex. Baylis (1932) and Joyeux & Baer (1936) include *T. ruudi* in their lists of parasites from Cetacea.

The four specimens collected in the present instance are all considerably shorter than those of Nybelin. His measurements were, respectively, 145, 180 and 183 mm., whereas the specimens given to the writer measured 61, 72, 80 and 132 mm. in length. The maximum breadth of the strobila is 2·5 mm. and the diameter of the scolex 1·5 mm., both of which measurements agree with those of Nybelin.

4. *Tetrabothrius wilsoni* (Leiper & Atkinson, 1914), Baylis 1926

SYNONYM. *Oriana wilsoni* Leiper & Atkinson.

HOST. *Physeter catodon* L. (Sperm whale, Cachalot).

OCCURRENCE. One specimen from a 55 ft. male Sperm whale; Ross Sea area; 20 January 1952.

Oriana wilsoni was described by Leiper & Atkinson (1914) from *Balaenoptera borealis* captured off the Bay of Islands, New Zealand. The generic characters given were 'Cyclophyllid with large quadrate discoidal head carrying four rounded suckers. Rostellum absent.' The species was described as 'Segments all immature. Strobila 13 cm. Head discoidal, 3 mm. in diameter, quadrate in outline, 4 rounded suckers present terminally. Neck very slender. Testes arranged in two definite groups of 7–8 and 17–18. Near to Diplobothrium.' A further brief description was later given by the same authors (1915), including three figures, two of the scolex and one of the immature proglottides. Baylis (1926) considered the genus *Oriana* to be synonymous with the genus *Tetrabothrius*, an examination of the co-type material at the Natural History Museum showing that 'the scolex is of precisely the same type as that of *T. affinis*'. All specimens of this worm so far discovered have been immature, and little is known of the anatomy of the strobila. The segments figured by Leiper & Atkinson (1915) were about the 221st and 222nd and show the genital pore opening laterally with a straight vas deferens and vagina running from it towards the centre of the segment. There are two groups of rounded testes, the poral groups including 7–8 and the aporal 17–18. There is no trace of female genitalia other than the vagina. It is possible that the species attains a considerable length when fully grown and that the development of the genitalia is slow. Presumably no specimen long enough to include fully mature segments has yet been discovered.

Baylis (1932) included the species in his list of parasites of Cetacea, and Baer (1932) refers to it, mentioning that there are 45–50 testes, considerably more than the 24–28 previously recorded.

In the present instance *T. wilsoni* is recorded for the first time from *Physeter catodon*, the only host previously known being *Balaenoptera borealis*. Only one specimen was found, measuring 300 mm. in length, about twice the length of the specimens recorded by Leiper & Atkinson. The maximum breadth of the strobila is 1·5 mm. and the diameter of the scolex 3 mm. The anatomy agrees in all respects with the original description, and even in the last segments the condition of the genitalia was still immature and similar to that described by Leiper & Atkinson.

The occurrence of both *Tetrabothrius wilsoni* and *Priapocephalus grandis* in a toothed whale is interesting, as previously both had been recorded only from baleen whales. The food in the two types of whale is very different—and a knowledge of it may be of assistance in an attempt to determine the life histories of these species.

5. *Tetrabothrius* sp.

An unidentified species from the intestine of *Balaenoptera musculus* L. (Blue whale, Sulphur-bottom, Sibbald's rorqual). This species was found on two occasions, namely, on 19 January 1952, when five specimens were obtained from an 83 ft. female Blue whale, and on 20 January, when sixteen specimens were taken from an 87 ft. female Blue whale. In both cases the parasites were located in the small intestine, in the former instance the infestation was slight and in the latter much heavier.

This species does not seem to have been described or recorded previously, but it does apparently occur in the material from the *Discovery* Expedition which is now in the Natural History Museum. Dr Markowsky, in his revision of the cestode parasites of Cetacea, will doubtless describe and name

this species should it prove to be new. Only a brief mention, therefore, is made of it here.

The specimens vary very much in length. The smallest immature specimen was 5·1 mm. in length and the longest fully developed worm 140 mm. They can be grouped according to their length as follows: two specimens below 10 mm., five between 20 and 40 mm., seven between 60 and 100 mm. and seven over 100 mm. The average length for the twenty-one specimens is 73 mm. The maximum breadth of the strobila in the larger specimens is 2 mm. and the diameter of the scolex is also 2 mm. The scolex is typical of the genus *Tetrabothrius* with four large suckers, 'auricular appendages' and 'epaulettes'. There are from 45 to 55 testes per segment, measuring 0·083 × 0·062 mm. to 0·117 × 0·052 mm. The cirrus sac measures 0·19–0·20 × 0·13–0·14 mm. The reproductive ducts pass ventrally to the excretory vessels, and the eggs measure 0·020 × 0·016 mm.

NEMATODA

Family Ascaridae

1. *Anisakis physeteris* Baylis, 1923

Host. *Physeter catodon* L. (Sperm whale, Cachalot).

Occurrence. A number of specimens from the stomach and intestine of three Sperm whales; Ross Sea area; 9–20 January 1952. The details of infestation are as follows:

Whale 1, 9 January: one mature female and several immature specimens from the intestine of a 53 ft. male Sperm whale. Total infestation slight.

Whale 2, 10 January: two males and five females from the first stomach of a 51 ft. male Sperm whale. Infestation heavy.

Whale 3, 16 January: four females from the intestine of a 55 ft. male Sperm whale. Infestation slight.

Mr Ross Cockrill states in some notes given to the writer that infestation of the stomach of Sperm whales with this species is invariably very heavy and that the weight of parasites in the first portion of the stomach may approximate 100 lb.

Anisakis physeteris was first described by Baylis (1932) from the stomach of *Physeter catodon*. The description was a detailed one and a comparison with five other species of the same genus from various cetacean hosts is included in the form of a table. Baylis indicates that *Anisakis physeteris* differs from the others in being of stouter build, the fineness of the cuticular striations, the form of the ventriculus which is straight and not sigmoid in shape, the extremely short spicules and the relatively large eggs. Baylis (1929) mentioned the species again in his account of parasitic Nematoda and Acanthocephala collected during the *Discovery* investigations between 1925 and 1927 at Durban, Saldanha Bay, South Africa, and South Georgia. This collection proved to be of great interest as it included five new species of nematodes and three of Acanthocephala, it also extended our knowledge of the distribution of many other forms in Antarctic and subantarctic waters. Baylis (1932) again included *A. physeteris* in his list of parasites from Cetacea. It has not been recorded previously from the intestine of the host; the stomach seems to be its normal site, but in the present instance it has been found twice in the intestine.

No further description will be given here as the specimens agree in every particular with the original description. Some of the female specimens found in the present instance have a greater length than that given by Baylis. They measure from 78 to 130 mm. in length, whereas Baylis gives the length as 80–112 mm. The structure of the lips and the arrangement of the oral papillae are identical in both sexes as also is the tail.

Family Spiruridae

2. *Crassicauda crassicauda* (Creplin, 1829), Leiper & Atkinson, 1915

Synonym. *Filaria crassicauda* Creplin, 1829.

Host. *Balaenoptera physalus* L. (Fin whale, Finback, Finner, Common rorqual).

Occurrence. Caudal extremities of five specimens, four males and one female, from the ureter of a Fin whale; Ross Sea area; 12 January 1952. Portions of infested kidney of two Fin whales, 12 and 15 January.

The species was first described by Creplin in 1829 as *Filaria crassicauda*. Beneden (1870), Cobbold (1879) and Baylis (1916) state that Creplin's material came from a specimen of *Balaenoptera acutorostrata* Lacep. stranded off the island of Rügen in the Baltic in 1825. Baylis (1916) further stated that Munter, having examined the skeleton of the same whale, identified it as *B. musculus* L., and that Hamer was of the opinion that the whale in question was *B. physalus*. There seems, therefore, to be some doubt as to the specific identification of the host in which this parasite was first found. Diesing has recorded the host of Creplin's material as '*Balaena borealis*' and Linstow as *B. mysticetus* L. Both these records, according to Baylis (1916), are erroneous. *Crassicauda crassicauda* was first found in the northern hemisphere, but Leiper & Atkinson (1914) recorded it in the southern hemisphere during the *Terra Nova* Expedition from the renal tubules of *Megaptera nodosa* off New Zealand. Other records of it from the southern hemisphere have appeared since. Baylis (1916) listed five species of hosts for this worm, namely, *Balaenoptera physalus* L., *B. musculus* L., *B. borealis* Lesson, *Megaptera nodosa* Bonn. and *Ziphius cavirostris* (Cuvier's whale). The

first four of these hosts are baleen whales and the last a toothed whale. As Baylis suggests, this fact may be of use in determining the intermediate host (if any), as the food differs greatly in the two types. Hamilton (1916) records the parasite from twenty-two specimens of *Balaenoptera physalus*, one *B. musculus* and one *B. borealis*. Baylis (1920) obtained two sets of specimens from *B. musculus* from Deception Island, South Shetlands, which he identified as *Crassicauda crassicauda*. On re-examining the *Terra Nova* material at the Natural History Museum he concluded that there were two species of the genus. He gave a generic diagnosis, naming the genotype as *Crassicauda crassicauda* (Crepl. 1829) nec. *C. crassicauda* (Crepl.) of Leiper & Atkinson, 1914 and 1915, from *Balaenoptera physalus* and *B. musculus*. The other species, namely, that collected during the *Terra Nova* Expedition, he named *Crassicauda boopis* Baylis (*C. crassicauda* (Crepl.) of Leiper & Atkinson, 1914 and 1915) from *Megaptera nodosa* and doubtfully from *Ziphius cavirostris*.

Later, Baylis (1922) obtained further material of *Crassicauda crassicauda* from South Georgia, and the same author (1932) recorded the hosts as *Balaenoptera physalus*, *B. musculus*, *B. borealis* and *Tursiops truncatus*.

Crassicauda crassicauda normally occurs in the urogenital system of its host, Creplin's original specimens being recorded from the corpora cavernosa of the penis. Hamilton (1916) described the ureter of the Fin whale as traversing the kidney for the greater part of its length, receiving numerous branches which ramify through the kidney and which terminate in the calyces of the lobules. He found the posterior portions of the worms lying free in the kidney tubules, the anterior ends passing through the wall of the calyx into the interlobular tissue where they are embedded in a mass of connective tissue through which they pursue a tortuous course. Hamilton suggested that the presence of the nematode causes hypertrophy of the tissues in the region where it occurs, forming 'connective tissue masses' which invariably contain specimens of the worm. Baylis (1920) also described the worms as being found with their caudal ends hanging free in the urinary passages and the embedded portion taking so twisted a course that it is almost impossible to remove a complete specimen. Baylis (1922) obtained further material from Mr A. G. Bennett from South Georgia, together with notes and a sketch which gave a better idea of the burrowing habits of the worm. Mr Bennett stated that the 'connective tissue masses' observed by Hamilton in the kidneys of *Balaenoptera physalus* may also be found in other tissues. Baylis has included a diagram, which is an adaptation of Mr Bennett's sketch, which shows 'an excised portion of the penis of *Balaenoptera* with a specimen of *Crassicauda crassicauda in situ*'. The free caudal portion of the worm is seen hanging in the urethra, and the anterior portion, after passing through the dense fibrous sheath of the corpus cavernosum for a short distance, becomes much coiled and surrounded by a nodule. The substance of this nodule is described as putty-like in some cases and in others apparently calcareous. The anterior extremity of the worm passes on from this nodule to a second filled with pus and fibrous tissue in which the head lies. It is the coiling of the worms in these nodules which prevents them from being pulled out and which makes removal of a complete specimen by dissection extremely difficult. Mr Bennett, however, succeeded in removing an entire specimen, and the anterior portions of several others, which enabled Baylis (1922) to make further studies of the anatomy.

Descriptions of the species have been made by Baylis (1916, 1920 and 1922). Previous accounts gave little but measurements, e.g. Creplin's original specimens were described as male 6½ in. long and female 13 in. long. This material contained complete specimens but the drawings are apparently not very clear. Baylis (1916) described the anterior portion of a specimen which consisted of a spirally twisted fragment measuring 30 cm. in length. The head end tapers to a narrow extremity and the mouth is *Filaria*-like in appearance, being a dorso-ventral slit devoid of lips but with one small inner papilla and three large outer papillae on either side. A few years later, Baylis (1920) examined more material, consisting of fragments of the caudal extremities of both sexes. These measured up to 10·5 cm. in length and not more than 2 mm. in thickness. The tail of the male is laterally compressed and spirally coiled, and the cloaca 0·5 mm. from the tip is followed by a ventral groove. On either side of the groove is an irregular row of caudal papillae, up to twelve in number on the left and eleven on the right. In the female the vulva is 1·5–3·0 mm. from the tip of the tail and lies in a constriction which marks off a knob-like caudal extremity. The anus is in a depression at the posterior end. The vagina is very short and gives off two forwardly directed thick-walled uteri. The ova are thick shelled and contain a coiled embryo when laid. Baylis compared the species with *Crassicauda boopis*, diagrams of the tail of the male and female of both being given. It was, however, uncertain whether the head end described earlier belonged to *C. crassicauda* or *C. boopis*. In 1922 Baylis obtained a complete male specimen of *C. crassicauda* and was able to establish the fact that the head belonged to this species. So far no description exists of the anterior extremity of *C. boopis*; apparently no specimen has yet been dissected from the host tissues.

The specimens of *C. crassicauda* examined in the present instance consist of the caudal portions of four males and one female removed from the ureter of *Balaenoptera physalus*. The four male fragments measure, respectively, 96, 115, 140 and 154 mm. in length, with a breadth of 1·5 mm. The single female fragment measures 130 mm. in length and 2·5 mm. in breadth. In each case the tail was complete, and an examination showed that in both cases the male and female structure compared exactly with the description given by Baylis (1920). The number of caudal papillae in the male is variable, and in the four specimens they are: 7 right, 9 left; 8 right, 11 left; 10 right, 11 left; and 11 right, 9 left.

In addition to these specimens, the writer has received from Mr Ross Cockrill some portions of the kidney of Fin whales thought to be infested with *Crassicauda crassicauda*. One of these, collected on 15 January, was described as 'greenish areas of caseation in the kidney reniculi'. The material was carefully examined and was found to be heavily infested with *Crassicauda*. As the excised portions of the kidney were small the worms had been cut and only small fragments were present. No complete tails of either male or female could be found, but the diameter of the body agreed with that of *C. crassicauda*. It was impossible to estimate the number of worms present, but almost all the tubules contained fragments of from one to four specimens. There was no regular distribution of the sexes in any group, the individuals being either all males or all females or any combination of both. Whitish nodules were present in the interlobular tissue and attempts were made to trace the worms to these nodules. They could be followed for a short distance into the interlobular tissue, but as a result of preservation they were extremely brittle and broke very easily. Any attempt to remove entire the buried portions of the worms was unsuccessful and it is probable that fresh material would be necessary for the purpose. Some of the nodules were cut or teased open and were found to contain coiled portions of the worms similar to those figured by Baylis (1920). Another specimen, obtained on 12 January, was described as 'small calcifying lesions from the kidney of the Fin whale'. Here a fragment of only one specimen of *Crassicauda* was found in a urinary tubule but it was impossible to dissect out the buried portion.

Two other pieces of whale kidney were included in the series of specimens. One of these was described as 'hyperaemia in a portion of a secondary urinary duct of a Fin whale' and the other as 'portions of a calcifying root-like growth from the kidney of a Blue whale'. The writer has failed to find any specimens of *Crassicauda* in either of these or in lesions taken from other parts of the body.

ACANTHOCEPHALA

1. *Bolbosoma hamiltoni* Baylis, 1929

Host. *Balaenoptera physalus* L. (Fin whale, Finback, Finner, Common rorqual).

Occurrence. Five specimens, two males and three females, from the large intestine of a female Fin whale; Ross Sea area; 22 January 1952. Infestation fairly heavy.

Bolbosoma hamiltoni was first described by Baylis (1929), who found a single male and several females in a specimen of *Balaenoptera physalus* caught at South Georgia in 1926. A single specimen was also found in *B. musculus* from the same locality. The description given of this species is adequate for the purpose of identification. Baylis gave the length of the male as 60 mm. and the breadth 2·05 mm.; the two males recorded here measure, respectively, 45 and 46 mm., with a breadth of 2 mm. The females are described by Baylis as measuring about 64 mm. in length and 2·7 mm. in breadth, which compares with the present measurements of 50–64 mm. in length and 2·5 mm. in breadth. The measurements of the bulb and proboscis agree very closely, the proboscis being armed with 26 longitudinal rows of hooks, each row including 7 or 8. The bulb bears rows of numerous small spines which increase slightly in length posteriorly. The arrangements of the male and female organs have not been examined in detail, but the egg measurements agree with those given by Baylis. The species is again recorded by Baylis (1932) in his classified list of cetacean parasites.

The anterior extremity of *Bolbosoma hamiltoni* is deeply embedded in the intestinal wall, there being small raised areas of inflammation at the point of insertion. With care the worms can be dissected out, but the process is sometimes difficult as the penetration is not always directly at right angles to the intestinal wall, and the embedded portions of some specimens turn at an angle so as to lie parallel to the lining of the intestine. Large numbers of these worms undoubtedly cause much irritation and damage to the intestinal wall of the host.

2. *Bolbosoma porrigens* (Rud., 1814), Porta, 1908

Synonyms. *Echinorhynchus balaenae* Gmelin, 1790 e.p., *E. porrigens* Rud. 1814, *Bolborhynchus porrigens* Porta, 1906.

Host. *Megaptera nodosa* Bonn. (Humpback).

Occurrence. Twenty-one specimens from the large intestine of two Humpback whales; Ross Sea area; 2 January 1952.

Whale 1: twelve specimens, four males, five females and three specimens which have not been detached from the intestinal wall, from the reticulate large intestine of a 48 ft. female Humpback whale.

Whale 2: nine specimens, five males and four females, from the same region of a 42 ft. female Humpback whale.

Bolbosoma porrigens has been recorded from six species of whale hosts, namely, *Balaena mysticetus*, *Balaenoptera acutorostrata*, *B. borealis*, *B. musculus*, *Megaptera nodosa* and *Hyperoodon rostratus*. All the literature on the species has not been available to the writer but many accounts of it have been consulted. Dujardin (1845) and Beneden (1870) record it from *Balaenoptera rostrata* (= *B. acutorostrata*). Cobbold (1879) found it to be one of the commonest parasites of the same host in the Firth of Forth and also recorded it from *Balaena mysticetus*. Borgström (1892) has given a fuller account of the anatomy, together with some diagrams and measurements, and has recorded it from *Balaenoptera borealis*. He also included a key for the identification of three species of *Echinorhynchus* now referred to the genus *Bolbosoma*, namely, *B. porrigens*, *B. turbinella* (Dies.) and *B. brevicolle* (Malm.). Shipley (1899), in his notes on species of *Echinorhynchus* parasitic in Cetacea, mentioned that Westrumb recorded *Bolbosoma porrigens* from *Hyperoodon rostratus* and that Collet added the new host *Megaptera boops* (= *M. nodosa*). Baylis (1932) included the parasite in his catalogue of worms from Cetacea.

According to Borgström (1892) live specimens are brick red or orange red in colour or a paler variation of these. The preserved specimens examined in the present instance still retain a pinkish colour. The species can be recognized by the characteristic cone-shaped bulb 4·5 mm. in diameter which is flattened on its anterior face, a sharp margin separating this from the posterior region. The bulb is unarmed, but the retractile proboscis measuring 2 mm. in length bears 24 longitudinal rows of 6 or 7 hooks each. Following the proboscis is a long narrow 'neck' constituting about a sixth of the total body length. This widens gradually posteriorly to form the hinder part of the body, which continues in a uniform width to the posterior extremity. Dujardin (1845) gave the length as 27–160 mm. and the breadth 1·12–3·37 mm., a ratio of 41:1. Borgström's specimens measured 110 mm. in length and 4·0 mm. in breadth at the hinder end, a ratio of 25 : 1. In the present instance the longest female specimen measured 174 mm. and the longest male 128 mm. An average for a number of specimens, including males and females, was 101 mm. and the breadth 4·0 mm., a ratio again of 25:1, which agrees with Borgström's measurements.

When the worms are *in situ* the 'neck' is threaded through the honeycomb reticulate wall of the large intestine with only the broader part of the body hanging free. This species is even more difficult to detach without damage than the previous species, but with care they can be dissected out. The bulb is deeply embedded in the intestinal wall and it and the 'neck' run parallel to the lining of the intestine.

HOST LIST

(* New host records)

Balaenoptera musculus L. (Blue whale, Sulphur-bottom, Sibbald's rorqual).

Synonyms: *B. sibbaldii*, *B. intermedia*.

Cestodes: *Tetrabothrius affinis* (Lonnberg, 1892). *Tetrabothrius* sp.

Balaenoptera physalus L. (Fin whale, Finback, Finner, Common rorqual).

Cestodes: *Tetrabothrius ruudi* Nybelin, 1928.

Nematodes: *Crassicauda crassicauda* (Creplin, 1829).

Acanthocephala: *Bolbosoma hamiltoni* Baylis, 1929.

Megaptera nodosa Bonn. (Humpback whale).

Synonym: *M. longimana*, *M. boops*.

Acanthocephala: *Bolbosoma porrigens* (Rud. 1814).

Physeter catodon L. (Sperm whale, Cachalot).

Synonym: *P. macrocephalus*.

Cestodes. **Priapocephalus grandis* Nybelin, 1922. **Tetrabothrius wilsoni* (Leiper & Atkinson, 1914).

Nematodes: *Anisakis physeteris* Baylis, 1923.

APPENDIX

In addition to the parasitic worms the collection given to the writer included some external parasites, namely, three species of barnacles and the whale louse. Barnacles are apparently fairly common on whales, and according to Harrison Mathews (1952) they are for the most part found on the slower-moving species where the current of water passing over the surface is not sufficiently strong to dislodge the larval stages while they are in the process of settling on the host. The three species of barnacles are described by Darwin (1851) and the whale louse by Sars (1890–5).

Coronula diadema L.

HOST. *Megaptera nodosa* Bonn. (Humpback).

OCCURRENCE. Sixteen specimens from the 'rifler' in the throat region, the anterior part of the lower jaw and the flipper of three Humpback whales.

This species is easily recognizable, the shell being 'crown shaped with longitudinal convex ribs having their margins crenated; orifice hexagonal; radii moderately thick, very broad: terga absent or rudimentary'. The specimens varied in size, the

largest measuring 42 mm. in length, 36 mm. in width and 25 mm. in height. Average measurements for the whole sample were 27 mm. long, 23 mm. wide and 17 mm. high. This species stands out from the surface of the host's body and is not deeply embedded in the skin.

Coronula reginae Darwin

HOST. *Balaenoptera musculus* L. (Blue whale, Sulphur-bottom, Sibbald's rorqual).

OCCURRENCE. Nine specimens from the anterior region of the lower jaw of a Blue whale.

The shell in this species is considerably more depressed than in *Coronula diadema* and is partially buried in the skin of the host. The apex of the barnacle projects through a six-rayed star-shaped cut in the dark skin. The shell is conical in shape, straight-sided, and the orifice hexagonal. The longitudinal flattened ribs have crenated edges and their surfaces are striated. The radii are thin and the terga absent. The largest specimen measured 28 mm. long, 28 mm. wide and 11 mm. high. Average measurements for the nine specimens were length 23 mm., width 22 mm. and height 9 mm.

Conchoderma aurita (L.)

OCCURRENCE. Fourteen specimens attached to the shells of *Coronula diadema* on *Megaptera nodosa*. Eleven specimens were attached to one barnacle and three to another. This species is apparently very common, being found on ships' bottoms from all parts of the world, on slow-moving fish and associated with other barnacles of the genus *Lepas* and *Conchoderma*. According to Darwin this is a very variable species. The peduncle is long and is clearly marked off from the capitulum. The capitulum has two tubular ear-like appendages placed behind the rudimentary terga, the scuta are bilobed and the carina either rudimentary or absent.

Paracyamis boopis (Lütken)

HOST. *Megaptera nodosa* Bonn. (Humpback).

OCCURRENCE. Twelve specimens from the flipper of a Humpback whale.

Sars (1890–5) records numbers of specimens from *Megaptera boops* (=*M. nodosa*) off the coast of Finmark. It was found by him on various parts of the whale body, e.g. head, belly-furrows, sexual region and breast fins. He noted that it was generally associated with *Coronula diadema*, which is also the case in the present instance.

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SYLVATIC PLAGUE STUDIES

X. SURVIVAL OF RODENT FLEAS IN THE LABORATORY

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The natural conditions that govern the longevity of fleas are of interest to epidemiologists and public health workers in the field of plague for two reasons: (1) If these conditions—temperature, humidity, and nourishment—are totally unfavourable, control may be left to them and it would be unnecessary to institute expensive control measures. (2) There is a question of how plague reservoirs, the perpetuating phase of plague, are maintained in nature, particularly in areas where the rodent hosts spend several months of each year in hibernation. It is important to know how long various fleas of the sylvatic reservoir may live and remain infected with the plague bacillus.

Coincident with a study of the vector efficiency of various fleas collected from areas in California where sylvatic plague was present, an opportunity was presented to make a laboratory study of these fleas under varied ecological conditions. It was hoped that such a study would give some indication of the potential life of individual fleas, and that from this the potential length of life of a flea in its natural habitat might be deduced and that some further light might be thrown on the factors that influence longevity of the flea.

The effect of climatic conditions on a plague epidemic has been known for many years. The Indian Plague Commission (1908) reported the observation that plague epidemics were checked when the mean daily temperature exceeded 80° F. (26·67° C.), and especially when it reached 85–90° F. (29·44–32·22° C.). Brooks (1917) studied the influence of saturation deficiency and of temperature on the course of epidemic plague in India, and reached the conclusion that when the temperature rises above 80° F. (26·67° C.), and the saturation deficiency exceeds 0·3 in., plague epidemics are brought to an end. He believed that for each range of temperature there is a critical saturation deficiency. Goyle (1928) studied the vector efficiency of *Xenopsylla cheopis* and *X. astia* and concluded that vapour pressure affected their ability to transmit plague. At 68° F. (20° C.), and 0·6 in. saturation deficiency, *X. cheopis* did not transmit plague, and 0·3 in. saturation deficiency sufficed to check *X. astia* from carrying plague.

According to Ioff (1941) the length of life of the insect depends primarily on its loss of moisture. This, in the absence of feeding, depends on vapour pressure of the air, the combined effect of temperature and atmospheric humidity.

The adult flea is indifferent to humidity, or nearly so, even when low humidity and high temperature are combined, and probably under natural conditions water loss in the adult would be made up the next time it fed (Buxton, 1938).

Sharif (1949) found that for flea larvae the critical humidity varies with the temperature, and it was not less than 45 % relative humidity, for the three Indian species of *Xenopsylla*, at the temperature at which he maintained these flea larvae. The limits of favourable humidity were not defined by a simple physical factor, such as a saturation deficiency value, as this varied at different temperatures. Sharif found that the humidity at which the larvae were kept influenced the size of the resulting adults, the largest adults developing from flea larvae kept at optimum humidities. The moisture content of the diet and solid surroundings was of more importance to larvae than was the moisture content of the atmosphere.

The flea actually has two different habitats: the body of the host and the nest or burrow of the host animal. Therefore, to know the complete ecological conditions of the flea, both of these must be investigated. Climatic conditions of various habitats are vastly different. For example, the temperature in certain parts of the burrow systems of rodents may be more constant, that is, lower in summer and higher in winter, than on the surface. The variation in deep burrows during a 24 hr. period is very slight. Animals in the nest may raise the temperature slightly. In the winter, five gerbils increased the nest temperature by from 2 to 6° C. (Rall, 1939). The nest of the rodent in a burrow system may be considerably closer to the surface in the summer than in the winter.

There is adequate proof that for different species of fleas, different microclimates are necessary for optimal growth of the population and maximum

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survival of individuals. Investigations of the microclimate of rodent burrows have shown that certain climatic conditions are favourable for certain fleas.

Ioff's studies (1941) of the seasonal fluctuations of ground squirrel fleas indicate that most of the flea population in the nests of this rodent changes annually. Most of the fleas did not survive more than 3–5 months, but a few lived much longer even in uninhabited nests and survived the winter. These studies in Russia of the flea populations of ground squirrel nests point out that at different seasons of the year different species of fleas are prevalent. In America, Stewart & Evans (1941) have shown that *Hoplopsyllus anomalus* is predominant on *Citellus beecheyi* in central California during the hottest time of the year, and *Diamanus montanus* is predominant at other times.

During the driest and hottest season the burrow temperatures of *Dipodomys spectabilis* ranged between 22·5 and 43·5° C. (Schmidt Nielsen & Schmidt Nielsen, 1950). Since these kangaroo rats cannot survive for any long periods at temperatures much above 38° C. in the laboratory, it was concluded that the holes where higher temperatures were recorded were probably not the main burrows. The moisture content of the air in the burrow was two to five times greater than that of the outside atmosphere; the relative humidity ranged from 17 to 62 %. Buxton (1932) found in a study of the microclimate of rat burrows, that the vapour pressure in the holes was nearly always higher than in the atmosphere outside. Even during July evaporation was occurring in the holes, and the difference in vapour pressure was considerably greater by day than by night.

In an artificial burrow constructed among a colony of *Citellus tereticaudus*, at 3 ft. vertical depth the mean minimum temperature was 72° F. (22·22° C.) and the mean maximum, 77° F. (25° C.) (Vorhies, 1945). Soil surface temperatures reached as high as 158° F. (70° C.) during the 1-week period in which the temperatures were studied. Over a 12-month period the highest burrow temperature reached was approximately 85° F. (29·44° C.) in July and August, and the lowest temperature recorded was approximately 55° F. (12·78° C.) in January and February.

In a study of the microclimate of *Neotoma albigula*, another animal found in desert areas of the south-western United States, a 12-month temperature record of the nest was made. The minimum temperature was about 49° F. (9·44° C.) in December and January and the maximum temperature was approximately 87° F. (30·56° C.) in early June.

The temperature conditions in gerbil burrows in the sandy semi-desert between the lower parts of the Volga and the Ural rivers have been studied (Rall, 1939). The temperature of the burrow did not differ more than 1° C. from that of a corresponding layer of sand. In shallow empty burrows at about 60 cm. depth, temperature fluctuated between −2·3° C. in January and 24·6° C. in July. Temperature in deep winter burrows, at about 3 m., fluctuated from 7·1° C. in March to 16·7° C. in September.

In a study of the microclimate of *Citellus beecheyi* burrows in central California (Longanecker & Burroughs, 1952) at a depth of 13 in. the highest temperature, 71·5° F. (21·94° C.), was reached in July and August, and the lowest, 44·5° F. (6·94° C.), in January. At a depth of 42 in. the highest temperature reached was 67·5° F. (19·72° C.) in late August, and the lowest was 50° F. (10° C.) in January and March.

These microclimate studies indicate the conditions to which the ectoparasites, which are not permanent parasites, are exposed when off the body of the host animal.

The question of the inter-epizootic reservoir of plague has also received considerable attention (Evseeva & Firsov, 1932; Golov & Ioff, 1925, 1926). The existence of plague-infected fleas through the winter has been proved many times (Golov & Ioff, 1926; Evseeva & Firsov, 1932). The maximum length of life in such fleas was 396 days, a time greatly exceeding the period between epizootic seasons. Tinker & Stupnitsky found plague-infected fleas in an uninhabited suslik nest in February, some days before the usual emergence of this animal from hibernation (Ioff, 1941). They believed that the overwintering of *Pasteurella pestis* in fleas was thus proved irrevocably. Suslik fleas are known to feed on their hibernating host (Ioff, 1941). Evseeva & Firsov (1932) infected fleas in the laboratory on a ground squirrel with plague bacteraemia, carefully secured the fleas and some nest material in cotton bags, and buried them. One nest was buried at a depth of 1·25 m. from 24 September to 3 January. Of 325 fleas placed in this nest, 104 were alive after 3 months and 10 days, and 26 of the 104 were still infected with plague bacilli. A second nest was buried 75 cm. deep and was excavated on 16 April, 6 months and 23 days later. There were 11 infected fleas among the 138 live fleas in the nest. On repeating the experiment, infected fleas were recovered 7 months and 11 days after being buried.

Under natural conditions in the Bombay hot weather of April to July, individual starved fleas, *Xenopsylla cheopis* and *X. astia*, lived as long as a week, but the majority died in 2–4 days (Webster, 1930). Some starved laboratory-bred fleas survived only 2–3 weeks at a temperature of over 80° F. (26·67° C.).

The factors that govern the length of time fleas of many different species can survive have been studied in laboratories in England, Africa, the

United States, Russia, India and other places. Temperature, humidity and feeding have been controlled and the length of life of the fleas has been recorded. The over-all approach of most of these studies is similar: different conditions of feeding, temperature and humidity are provided and the length of time the fleas live under these conditions is observed in an effort to determine the effects of environmental factors on the longevity of the flea.

An extensive pioneer study of the bionomics of fleas was made by Bacot (1914) who studied the effects of temperature and humidity on the various stages of common rat fleas and other species associated with human habitations. Perhaps the most interesting part of this work is the longevity data which Bacot obtained for adult fleas.

Bacot observed the following maximum longevities for fleas in his bionomic studies:

	Maximum length of life, days	
	Unfed	Fed periodically
Pulex irritans	126–130	513
Nosopsyllus fasciatus	Approx. 95	106
Xenopsylla cheopis	38	100
Ctenocephalides canis	58	345
Ceratophyllus gallinae	127	224

Many other workers have contributed to the knowledge of this subject with studies of the biology of fleas and the effects of climatic conditions on their longevity (Mellanby, 1933; Sikes, 1931; Leeson, 1932, 1936; Sharif, 1937; Nicoll, 1912 and others). Edney (1945, 1947*a*,*b*) explained the conflicting results obtained by some of the earlier investigators. For the investigation of the bionomics of fleas, Edney's findings are fundamental. His fleas were starved after being reared under various ecological conditions, and after being fed once, several times or not at all. The species studied were *Xenopsylla cheopis* and *X. brasiliensis*. His conclusions which concern the present study were that if blood which is to be powdered for larval food is dried in an oven at high temperatures, the blood proteins are denatured and such treatment lessens its nutritional value. Therefore, the larvae have nutritional deficiencies and adults which develop from such larvae do not live as long. The effect of the age of the parent flea was unmistakable too, as offspring of adults less than 10 days old outlived offspring of adults over 11 days old. Rearing many larvae together rather than separately adversely affected longevity also, as did keeping the larvae in a substratum of fine sand. Fine sand either adsorbed or scratched the waxy covering of the epicuticle so that the water reserve was not so great as in fleas reared in a substratum of coarser particles.

For the prepupae Edney found a critical relative humidity somewhere between 45 and 50%, below which they die and above which they absorb water. The quantity of water absorbed was independent of the relative humidity within the range of 50–90%. The quantity of water absorbed by the prepupae was approximately equal to the total increase in body weight.

The effect of pupal environment was unmistakable also, and the longevity of adults in Edney's work varied according to the humidity during the cocoon stage. The variation was due to loss of water during the pupal, not the prepupal, period. Adult fleas developing from pupae maintained at higher humidities weighed more, had a greater water content, and survived longer when subsequently subjected to starvation.

Newly emerged unfed adult males and females lived about the same length of time. With laboratory-reared specimens, unfed fleas outlived those that were fed once, and fleas fed once outlived those that were fed several times, if they were maintained at the same temperature and relative humidity and subjected to a period of starvation.

Perhaps the first observation of the survival of starved fleas for periods longer than those fed and then starved was made by the Indian Plague Commission (1908). Their laboratory-reared fleas never fed as adults outlived fleas taken from hosts when both types of fleas were starved under the same conditions in the laboratory. *X. cheopis* fed on either man or rats survived considerably longer on the blood of its natural host than on the blood of man. Mitzmain (1910), in studying the bionomics of some California rodent fleas, found that on a diet of human blood the females of all species studied outlived the males by several weeks. Fleas not fed during the adult stage and kept under the same conditions as fleas taken directly from the host lived longer. Hirst (1923) noted that once fleas have fed on blood they must have regular blood meals or perish. Hirst also considered it important that fleas placed in glass tubes be given material to rest on.

Maintaining a group of fleas in a single container increased the activity of the fleas and thus curtailed the life span by nearly half (Hirst, 1927). In studying the biology of *Nosopsyllus fasciatus*, Strickland (1914) found that in the presence of a quantity of substratum of somewhat hygroscopic rubbish, many unfed fleas were alive after 17 months when the experiment was discontinued. For a part of the experiment at least, a temperature of 60° F. (15·56° C.) and a relative humidity of 70% were maintained. Constant temperatures were not maintained throughout, however, for Strickland stated that many fleas died during the hot summer. At the temperatures of Strickland's experiments it is highly dubious if unfed adults could live so long, and

emergence of new fleas from immature stages in the rubbish must be the explanation for such longevity, if none were introduced during the experiment.

Fleas studied by Tiflov and Ioff (1932) lived far longer than those studied by any other investigators. They kept fleas in three different states of nutrition: (1) fed periodically, (2) fed once and (3) unfed. Two different physical environments were selected and each group of fleas was represented in each environment. The first was a cabinet where the temperature varied from 13 to 24° C. The other was a cellar where the temperature varied from −2 to 15° C. The fleas were kept in tubes of moist sand, and wet rags were hung in the vicinity to keep the relative humidity at 90–92%. Daily temperature changes did not exceed 1° C.

In their first series of experiments, the unfed fleas in the cellar, the maximum longevities were: *Ctenophthalmus orientalis*, 396 days; *C. breviatus*, 392 days; *Neopsylla setosa*, 350 days; *Ceratophyllus tesquorum*, 267 days; *Oropsylla silantiewi*, 256 days. In their second series, the fleas were fed only once and kept under the same conditions as the first group. In the cellar the average longevity was greater, but the maximum dropped from 369 to 353 days. The third group of fleas was fed periodically, usually at monthly intervals, but there were several lapses of 2 months, and once a lapse of 5 months during the winter and spring. The fleas were taken into the laboratory where, at temperatures of from 15 to 24° C., they were given opportunity to feed for 10–15 min. on shaved abdomens of susliks, guinea-pigs or white rats. The maximum longevity data obtained in the third experiment were: *Neopsylla setosa*, 1725 days; *Ctenophthalmus orientalis*, 715 days; *Ceratophyllus tesquorum*, 377 days; *Ctenophthalmus breviatus*, 342 days; *Oropsylla silantiewi*, 302 days; *Frontopsylla semura*, 194 days; *Mesopsylla hebes*, 42 days; *Nosopsyllus fasciatus*, 41 days; *Ctenopsylla musculi*, 37 days. In comparing these data with those obtained by other workers it becomes obvious that the conditions of these experiments were much better for the fleas.

MATERIALS AND METHODS

Sources of stock for the colonies of fleas

The source of the fleas and the means of maintaining them have been described (Burroughs, 1947), but since Edney has shown the importance of rearing methods, attention must be given to some details omitted from the previous publication.

Eight species of fleas, all taken from rodents known to contract plague under natural conditions, were used in these longevity studies: (1) *Xenopsylla cheopis* (Roths., 1903) and (2) *Nosopsyllus fasciatus* (Bosc., 1801) from Norway rats trapped on one of the dump grounds of the City of San Francisco; (3) *Opisodasys nesiotus* (Augustson, 1931) and (4) *Malaraeus telchinum* (Roths., 1905) from field mice in Marin County, near San Francisco Bay; (5) *Orchopeas sexdentatus sexdentatus* (Baker, 1904) from wood rats at the Hooper Foundation Field Station for Ecological Studies at Calaveras Dam, Alameda County, California; (6) *Diamanus montanus* (Baker, 1895) from California ground squirrels, live trapped at the same site; (7) *Megabothris abantis* (Roths., 1905) from *Microtus longicaudus* trapped in the Sierra Nevada Mountains near Lake Tahoe; and (8) *Oropsylla idahoensis* (Baker, 1904) from *Citellus beldingi* in the same area.

Conditions

All fleas used were laboratory bred. *Oropsylla idahoensis* and *Diamanus montanus* were maintained on ground squirrels, usually *Citellus beecheyi* (*C. beldingi* and *C. townsendi* were used at times); *Diamanus montanus* on white laboratory rats; *Malaraeus telchinum*, *Megabothris abantis* and *Opisodasys nesiotus* on *Microtus*; *Orchopeas sexdentatus sexdentatus* on both laboratory rats and *Neotoma*; *Xenopsylla cheopis* and *Nosopsyllus fasciatus* on white laboratory rats.

The fleas and host were housed in 5-gallon earthenware crocks with glazed inner surface. About 2 in. of wood shavings were placed in each crock to serve as substratum. Powdered horse or sheep blood, dried in small quantities in flat pans in a bacteriological incubator set at 37° C., was used for food. Dried, ground brewer's yeast, as recommended by Sikes (1931) was also used. Approximately equal parts of the dried blood and brewer's yeast, about 1 or 2 teaspoons of each, were added to the shavings in each crock. The mixture in the crock was then moistened and the host and fleas were placed in the crock. A piece of heavy hardware cloth was secured to the top of each crock to prevent the host animal from escaping.

The temperature of the room where the fleas were reared was maintained at 70–75° F. (21·11–23·89° C.) and the relative humidity was 80 to 90%. The humidity was maintained by hanging a cloth water-bag in the room. At times additional moisture was provided by sprinkling water on the concrete floor. A light was frequently left burning in the small rearing room to help maintain the temperature.

The length of time an animal was kept in a crock depended on the amount of urine produced, as excess of urine on the shavings harms the larvae, and the ammonia produced by bacterial action on the urea may become sufficiently concentrated to kill them. Another factor determining the length of time that a host remained in the culture crock, particularly those species that do not produce excessive quantities of urine, such as *Microtus* and *Neotoma*,

was the number of fleas produced in the crock. Under the conditions of rearing, cultures frequently contained such tremendous numbers of fleas that it was necessary to remove the host to prevent its death by exsanguination. A culture was then started with fewer fleas.

When fleas were needed for an experiment, a crock was selected in which adults were obviously emerging. The shavings and other substratum were transferred, a small handful at a time, to a white enamelware pan and spread out thinly over the bottom. All of the adult fleas were removed then with the aid of a suction pump. Each bit of substratum was transferred to a clean crock after all adult fleas had been removed. The adult fleas taken from this hand-picked substratum on the following day were then all considered to be 1-day-old fleas for the purpose of the experiment.

Three different types of containers were used for holding fleas taken from the colony for experiments. The first was a glass tube, 1 × 8 in., open at each end. A one-hole rubber stopper with a glass tube, the inside end of which was covered with fine gauze, was used to plug each end of the holding tube. In the early work a small quantity of wood shavings, first allowed to come to equilibrium with the humidity of the atmosphere, was placed in each tube. Later the shavings were replaced with a strip of filter-paper for convenience of handling fleas. Usually approximately fifty fleas were placed in each glass tube; the number varied considerably at times, from as few as approximately thirty to as many as approximately 100 in a tube. As far as could be seen the larger number of fleas did not shorten longevity with tubes of this size. Fleas were fed by removing them from the wood shavings or filter-paper and placing them in a larger tube held firmly against the shaved abdomen of the host by a supporting heavy wire upright. The host animal was restrained in a heavy wire-mesh cylinder.

The other two types of containers consisted of wood cover-slip boxes, 1¾ in. in diameter by 2 in. high, and paper pill-boxes, 1⅛ in. in diameter by ⅝ in. high. The open ends of the boxes were covered with silk gauze. This retained the fleas, but allowed them to feed through the mesh. As the fleas were fed, the host animal was secured to a feeding board, ventral side up, and the gauze surface of the container was taped to the shaved abdomen.

The flea containers were kept in humidity chambers, which in turn were kept in temperature cabinets. The humidity chambers were white enamelware refrigerator pans, and when in use the tops were sealed on with heavy coatings of petroleum jelly. The desired humidity was maintained with saturated solutions of appropriate salts. At first, while fleas were being kept in glass tubes, the salt solution was kept in a 250 ml. beaker placed in each pan. This was not satisfactory, as the surface was not sufficient to allow rapid enough evaporation from the solution, so instead the entire bottom of the pan was covered to a depth of ¾ to 1 in. with the salt solution. The flea containers were supported above the solution on a wire frame.

The temperature cabinets, obtained from the Precision Scientific Laboratory Supply Company, were converted electric refrigerator boxes equipped with Sunbeam refrigerating units, a rod heating-element, a constantly revolving fan to prevent stratification of layers of air of different temperatures, a bimetal micro-switch which served as a thermostat, and a temperature control dial. Before starting an experiment the appropriate temperature was maintained for a 24 hr. period, as indicated on a continuous recording thermograph. At times the micro-switch stuck and kept the refrigerating unit operating for several hours, or until the fleas were examined on the following day. Then the temperature fell far below the holding temperature of the experiment. The long-range effect on the experiment is not known, but there is no perceptible immediate effect such as unusual mortality.

The period of feeding varied from 15 to 60 min. and no routine check was made to determine if all fleas given the opportunity to feed had actually fed. It is readily seen that the conditions of feeding varied considerably between the fleas kept in glass tubes and those kept in boxes. The former fed only in the light, the latter in the dark. The fleas kept in glass tubes had direct access to the skin of the host, while those held in boxes were kept off the skin of the host by the gauze used to retain them in the box.

Other workers have reported that newly emerged fleas feed only occasionally and usually evince little or no hunger. When newly emerged fleas were removed from a breeding crock for an experiment, they were at the most 24 hr. old and were considered to be of this age. In only a few instances were they fed on the day they were removed from the crock. Usually they were fed on the next day, or when they were at most 48 hr. old. Some were fed on the second day after selection for the experiment, or at a maximum age of 72 hr., and a very few were fed for the first time at 96 hr., or on the third day after removal from a crock. The number of dead fleas was recorded daily by sex except on Sunday. The day they were found dead was recorded as the day of death.

EXPERIMENTAL WORK

The frequency distributions for longevity of the various groups are not given in this paper because of space limitations, but a summary of their characteristics will be given. The form of distribution shown by these data is most irregular. In some instances the distributions were compact and had

a near enough approach to symmetry to allow the usual type of significance tests based upon normal distribution theory. In many cases, however, the distribution was widely scattered, with peaks at the lower end of the scale, and sometimes also at other points, so that it was obviously impossible to use tests based upon normal distribution theory. The means of standard deviations are not given because of this gross anormality. It seems more appropriate to use median length of life as the typical value and base conclusions upon what appears to be correct, rather than to make elaborate calculations not justified by the nature of the data.

Xenopsylla cheopis (Tables 1 and 2)

Data in Table 1 reveal the following facts: when they were fed daily, the life of *X. cheopis* was somewhat shorter at 5 and 13° C. than at 20° C. The adverse effect of lowered moisture content of the air on longevity is evident throughout each group.

Table 1. *Data on the longevity of* Xenopsylla cheopis

				Males			Females			Total	
Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Median survival (days)
Glass tubes:											
Fed daily on guinea-pig	20	15	13·90	49	46	15	27	46	5	76	10
	20	56	7·72	59	49	5	23	47	16	82	7
	20	92	1·40	84	72	11	66	77	14	150	12
	13	15	9·55	17	4	3	32	6	3	49	3
	13	65	3·93	23	8	5	26	8	3	49	5
	13	94	0·67	17	10	7	32	31	8	49	8
	5	40	3·92	30	18	9	22	18	10	52	10
	5	95	0·32	30	19	13	20	19	12	50	13
Fed once on guinea-pig	20	56	7·72	38	13	8	4	11	2	42	8
	20	92	1·40	48	14	10	3	12	10	51	10
	13	15	9·55	19	8	5	42	8	5	61	5
	13	65	3·93	25	9	5	29	7	5	54	5
	13	94	0·67	29	11	7	26	12	7	55	7
	5	40	3·92	24	17	10	26	12	8	50	8
	5	95	0·32	28	18	15	22	16	13	50	13
Not fed	29	60	12·02	28	8	5	20	5	5	48	5
	20	56	7·72	62	13	7	8	9	5	70	7
	18	12	13·62	30	9	8	20	7	5	50	7
	18	56	6·81	33	14	11	19	8	9	52	10
	18	92	1·24	35	10	9	16	8	5	51	8
	13	15	9·55	15	7	6	24	12	6	39	6
	13	65	3·93	24	6	4	30	6	4	54	4
	13	94	0·67	18	13	8	37	13	8	55	8
	5	40	3·92	19	13	10	22	13	4	41	8
	5	95	0·32	26	19	14	15	14	12	41	13
Held individually in glass vials and not fed	20	92	1·40	59	24	11	75	26	7	134	9
	10	94	0·55	41	34	6	19	34	8	60	7

Table 2. *Data on the longevity of* Xenopsylla cheopis

				Males			Females			Total	
Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Median survival (days)
Pill-boxes:											
Fed daily on rat	20	92	1·40	35	24	11	88	158	14	123	12
Fed once on rat	21	92	1·49	45	14	8	19	11	5	64	8
	10	94	0·55	58	41	14	14	18	10	72	14
Not fed	21	92	1·49	44	15	10	10	8	4	54	10
	10	94	0·55	53	40	17	19	18	10	72	14
Wood boxes:											
Fed daily on rat	10	94	0·55	61	25	12	40	31	7	101	10

There is little difference between the longevity of males and females under corresponding conditions. The consistently longer survival of fleas at 5 than at 13° C. at the highest relative humidity must be explained by some factor other than the slight difference in saturation deficiency. One possible explanation is that at the lower temperature the

flea was almost or entirely dormant, while at 13° C. it was not dormant and the temperature was sufficiently high to allow physiological processes to function properly.

The longest-lived fleas were among those fed daily and kept at 20° C., and 92 % relative humidity; a male lived 72 days and a female 77 days.

Fleas kept singly in vials, unfed, outlived those at the nearest corresponding temperature and relative humidity kept together in numbers of 30–100. The unfed and the once-fed groups kept under similar conditions had very nearly the same maximum and median longevities; in any case they were too close to allow the effect of having or not having a single blood meal to be seen.

greater maximum longevity at 20° C. at all three conditions of saturation deficiency than under the same conditions of temperature and relative humidity when fed only once or when not fed. Differences in saturation deficiency had little if any effect on maximum longevity when the fleas were kept at 20° C. and fed daily.

The longest-lived fleas of those kept in glass tubes were a male fed daily and held at 20° C. and 15 % relative humidity, and a female fed daily and kept at 20° C. and 92 % relative humidity. Both survived for 50 days.

The longevity of *N. fasciatus* was affected by the daily opportunity to obtain a meal of rat blood more than any other factor of the experiment. The upper

Table 3. *Data on the longevity of* Nosopsyllus fasciatus

				Males			Females			Total	
Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Median survival (days)
Glass tubes:											
Fed daily on	20	15	13·90	78	50	7	91	47	8	169	8
guinea-pig	20	56	7·72	80	41	4	79	42	4	159	4
	20	92	1·40	84	47	6	69	50	8	153	7
Fed once on	20	15	13·90	81	11	8	92	13	8	173	8
guinea-pig	20	56	7·72	90	8	5	69	9	5	159	5
	20	92	1·40	74	11	7	81	11	6	155	6
Not fed	20	15	13·90	45	11	7	116	11	6	206	6
	20	56	7·72	106	10	3	113	8	2	219	3
	20	92	1·40	67	11	6	85	9	5	152	5
Pill boxes:											
Fed daily on rat	20	92	1·40	20	117	27	20	281	50	40	35
	10	94	0·55	24	6	2	13	43	4	37	4
Fed once on rat	20	92	1·40	25	13	7	22	9	6	47	7
	10	94	0·55	27	22	4	10	11	2	37	4
Not fed	20	92	1·40	34	13	4	11	5	3	45	3
	10	94	0·55	38	13	5	9	4	3	47	4

In Table 2, the longest-lived *X. cheopis*, a female, survived for 158 days when given daily opportunity to feed and kept at 20° C. and 92 % relative humidity. Of interest is the fact that the medians for the males and females fed daily, shown in Tables 1 and 2, are identical at 20° C. and 92 % relative humidity. Under these conditions, males and females kept in glass tubes had about the same maximum survival time, but the longest-lived females kept in pill-boxes lived over twice as long as the longest-lived individuals kept in glass tubes. The analysis of the experiment was complicated by the fact that the longest-lived male kept in a glass tube survived three times as long as the longest-lived male kept in a pill-box, when the fleas were fed daily and kept at 20° C. and 92 % relative humidity.

Nosopsyllus fasciatus (Table 3)

Both male and female *N. fasciatus*, when given opportunity to feed daily on a guinea-pig, had limits of survival, 117 days for males and 281 days for females, were reached at 20° C. and 92 % relative humidity when the fleas were given access to a rat daily, and were kept in pill-boxes. Median longevities of males and females were also much greater under those conditions. The fleas given one opportunity to feed and kept in pill-boxes held at 20° C. and 92 % relative humidity survived slightly longer than the unfed group. Groups of fleas kept at 10° C. and 94 % relative humidity, contained considerably longer-lived individuals, but the medians were about the same, favouring slightly the unfed groups.

Diamanus montanus (Tables 4 and 5)

The upper limits of longevity of *D. montanus* were 258 days for males and 331 days for females. The longest-surviving fleas were kept in pill-boxes at 21° C. and 92 % relative humidity and given opportunity daily to feed on a rat.

When these fleas were fed daily on their natural

hosts, *Citellus beecheyi*, the maximum survival time was not so great (Table 4), and it must be inferred that conditions in glass tubes were not as beneficial to the flea as were those in pill-boxes. However, the median survivals were longer when individuals were fed daily on *C. beecheyi* than when fed daily on either a rat or a guinea-pig. In all instances but one, the maximum survivals of fleas kept in glass tubes were greater when squirrels were used as hosts. The exception was a female fed on a guinea-pig and kept at 20° C. and 56 % relative humidity which lived for 101 days.

Comparing the groups given a single opportunity to feed and those given no opportunity to feed, the longest-lived individual was a female in the former group, which lived for 52 days when kept in a pill-box at 10° C. and 94 % relative humidity.

Table 4. *Data on the longevity of* Diamanus montanus

Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	Males: No. of fleas	Males: Maximum survival (days)	Males: Median survival (days)	Females: No. of fleas	Females: Maximum survival (days)	Females: Median survival (days)	Total: No. of fleas	Total: Median survival (days)
Glass tubes:											
Fed daily on guinea-pig	20	15	13·90	29	14	4	35	62	4	64	4
	20	56	7·72	15	30	15	27	101	15	42	15
	20	92	1·40	24	42	15	68	83	22	92	17
	13	15	9·55	53	14	6	50	15	5	103	5
	13	65	3·93	36	13	11	46	15	11	82	11
	13	94	0·67	36	22	6	62	37	5	98	5
	5	40	3·92	25	12	7	24	12	6	49	7
	5	95	0·32	59	10	5	52	11	4	111	5
Fed daily on ground squirrel	18	35	10·06	20	67	33	17	74	46	37	37
	18	56	6·81	25	62	29	16	63	53	41	31
	18	92	1·24	24	46	21	22	85	32	46	23
Fed once on guinea-pig	20	56	7·72	22	10	6	22	12	4	44	5
	20	92	1·40	27	11	9	22	14	10	49	9
	13	15	9·55	39	10	7	67	14	7	106	7
	13	65	3·93	35	13	11	65	21	10	100	10
	13	94	0·67	37	14	6	46	15	8	83	7
	5	40	3·92	30	13	8	35	12	5	65	6
	5	95	0·32	54	13	3	72	6	3	126	3
Fed once on ground squirrel	18	35	10·06	25	16	12	29	17	12	54	12
	18	56	6·81	10	16	15	40	19	14	50	14
	18	92	1·24	11	18	6	37	19	14	48	13
Not fed	18	12	13·62	28	9	6	26	11	6	54	6
	18	35	10·06	19	16	14	27	18	14	46	14
	18	56	6·81	22	19	15	30	20	16	52	15
	18	92	1·24	21	22	17	30	22	17	51	17
	13	15	9·55	52	12	5	51	6	3	103	5
	13	65	3·93	45	14	8	60	16	12	105	10
	13	94	0·67	39	16	6	72	15	4	111	6
	5	40	3·92	29	15	5	22	8	4	51	5
	5	95	0·32	82	13	4	77	26	5	159	5

Table 5. *Data on the longevity of* Diamanus montanus

Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	Males: No. of fleas	Males: Maximum survival (days)	Males: Median survival (days)	Females: No. of fleas	Females: Maximum survival (days)	Females: Median survival (days)	Total: No. of fleas	Total: Median survival (days)
Pill-boxes:											
Fed daily on rat	21	92	1·49	25	258	9	62	331	24	87	20
	10	94	0·55	48	19	3	39	42	3	86	3
Fed once on rat	21	92	1·49	50	46	9	49	24	10	99	9
	10	94	0·55	38	28	4	41	52	3	79	4
Not fed	21	92	1·49	43	25	8	48	34	9	91	9
	10	94	0·55	42	34	3	48	45	2	90	2

A single meal of squirrel blood seemed to prolong the life of *Diamanus montanus* slightly more than did a single meal of guinea-pig blood. The effect of different temperatures and humidities was undoubtedly secondary to the effect of a single blood meal in this group of fleas as none survived for more than a few days, and median survivals varied in no

consistent manner in response to variations in saturation deficiency or temperature.

The unfed group kept in glass tubes had a maximal survival of 26 days; the flea that lived the longest was a female held at 5° C. and 95% relative humidity. The median and maximum longevity of fleas held at 18° C. was longer than those held at 5 or 13° C. The 11° C. temperature difference for fleas held in pill-boxes under all three nutritional conditions (Table 5) affected the median survival time. The median life span of fleas held at 21° C. was from 5 to 21 days longer.

Oropsylla idahoensis (Table 6)

This flea was kept only in pill-boxes or wood boxes. It is readily seen that the potential longevity of these fleas was great; when given daily opportunities to feed they lived from 6 months to nearly a year.

Table 6. *Data on the longevity of* Oropsylla idahoensis

Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	Males: No. of fleas	Males: Maximum survival (days)	Males: Median survival (days)	Females: No. of fleas	Females: Maximum survival (days)	Females: Median survival (days)	Total: No. of fleas	Total: Median survival (days)
Pill-boxes:											
Fed daily on rat	21	92	1·49	29	189	23	30	326	45	59	32
	10	94	0·55	8	46	26	14	54	39	22	30
Fed once on rat	21	92	1·49	25	31	22	23	36	21	48	21
	10	94	0·55	27	43	29	25	41	29	52	29
Not fed	21	92	1·49	25	31	21	18	33	20	43	21
	10	94	0·55	26	27	6	36	37	18	62	13
Wood boxes:											
Fed daily on rat	21	92	1·49	2	45	10	5	189	105	7	48
	10	94	0·55	17	236	15	18	352	44	35	20

This flea has a wide host range, but probably uses rats, voles or field mice for hosts only accidentally. That it was able to subsist very well on rat blood in the present studies is evident. Perhaps, as in the case of *Diamanus montanus*, longevity would have been greater if the blood meals had been obtained from a ground squirrel.

The effect of temperature on fleas given an opportunity to feed daily is very difficult to assess. Both the longest-lived male and the longest-lived female were in lots kept at 10° C., but in lots kept at 21° C. there were also long-surviving individuals, and the medians appear to slightly favour the temperature of 21° C.

Among those fleas fed once on a rat, a temperature of 10° C. seemed more favourable for survival. The longest-lived unfed flea was a female held at 10° C., but generally unfed fleas held at 21° C. survived longer than those held at 10° C. This was particularly so among the males.

Fleas held at 21° C. survived approximately the same length of time whether given a single blood meal or starved. Among the fleas held at 10° C., however, a single blood meal very noticeably prolonged the life of the males and less so of the females.

Opisodasys nesiotus (Table 7)

This was not a very long-lived flea in these experiments. Of those kept at 20° C. and fed daily on a guinea-pig, the median survival time was longest at the highest humidity and lowest at the intermediate humidity, though one female lived the longest period of any in this set at the intermediate relative humidity. At 13° C. humidity had little effect on longevity of fleas fed daily on a guinea-pig.

The fleas given one opportunity to feed on a guinea-pig died so rapidly that the effect of temperature and relative humidity cannot be seen. Fleas allowed access to a rat once also died very quickly; the median longevities do not reflect the influence of the various temperatures or relative humidities. The maximum longevity in this group, however, was among those kept at the highest relative humidity.

Of the unfed fleas held at 20° C., those at 92% relative humidity lived slightly longer than those at 15 or 56%.

A single blood meal prolonged the life of these fleas slightly, and this effect was more evident in the males.

Examining the data for *O. nesiotus* it is seen that the flea survived longer under all feeding conditions when kept in pill-boxes than when kept in glass tubes.

The effect of temperature is insignificant among the fleas fed daily or fed once. The starved fleas survived longer at the highest temperature.

Malaraeus telchinum (Table 8)

Male *M. telchinum* kept in glass tubes and fed daily did not live any longer at higher humidities; the longest-lived male survived for 38 days at the intermediate humidity. The greatest median survival time was 17 days at the lowest relative

Table 7. *Data on the longevity of* Opisodasys nesiotus

Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	Males			Females			Total	
				No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Median survival (days)
Glass tubes:											
Fed daily on guinea-pig	20	15	13·90	19	15	7	20	30	14	39	12
	20	56	7·72	26	9	5	30	38	5	56	5
	20	92	1·40	28	21	10	25	36	19	53	13
	13	15	9·55	15	7	3	19	7	3	34	3
	13	94	0·67	25	7	4	25	15	4	50	4
Fed daily on rat	20	15	13·90	14	20	3	44	39	19	58	13
	20	56	7·72	24	15	6	18	33	15	42	8
	20	92	1·40	26	7	6	23	23	6	49	6
Fed once on guinea-pig	20	15	13·90	29	8	5	25	9	6	54	6
	20	56	7·72	30	7	5	24	8	6	54	5
	20	92	1·40	42	7	6	61	9	5	103	6
	13	94	0·67	25	6	4	27	6	3	52	4
Fed once on rat	20	15	13·90	24	8	6	29	9	7	53	6
	20	56	7·72	24	6	6	29	8	6	53	6
	20	92	1·40	34	12	6	22	10	6	56	6
Not fed	20	15	13·90	52	7	4	45	7	5	97	4
	20	56	7·72	52	6	4	52	7	5	104	5
	20	92	1·40	57	8	4	51	9	6	108	6
	13	94	0·67	10	5	2	18	7	1	28	1
Pill-boxes:											
Fed daily on rat	20	92	1·40	33	47	3	16	43	2	49	3
	10	94	0·55	20	48	2	27	7	2	47	2
Fed once on rat	20	92	1·40	18	7	4	27	17	3	45	4
	10	94	0·55	19	6	4	27	8	4	46	4
Not fed	20	92	1·40	18	15	3	30	10	3	48	3
	10	94	0·55	14	5	3	30	5	2	44	2

Table 8. *Data on the longevity of* Malaraeus telchinum

Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	Males			Females			Total	
				No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Median survival (days)
Glass tubes:											
Fed daily on guinea-pig	20	15	13·90	38	29	17	13	34	17	51	17
	20	56	7·72	25	38	13	21	39	14	46	13
	20	92	1·40	25	25	12	21	42	26	46	19
Fed once on guinea-pig	20	15	13·90	22	9	7	23	12	9	45	7
	20	56	7·72	35	8	5	15	9	7	50	6
	20	92	1·40	29	12	8	21	12	9	50	8
Fed once on ground squirrel	18	35	10·06	19	11	10	32	15	12	51	11
	18	56	6·81	24	13	11	26	20	14	50	12
	18	92	1·24	22	16	12	26	17	14	48	13
Fed once on vole	18	35	10·06	32	11	9	21	11	10	53	9
	18	56	6·81	24	10	8	21	11	9	45	8
	18	92	1·24	38	14	8	15	12	8	53	8
Not fed	20	15	13·90	32	10	8	34	11	9	66	8
	20	56	7·72	20	9	6	28	9	6	48	6
	20	92	1·40	19	12	7	39	12	7	58	7
	18	35	10·06	35	11	8	11	12	10	46	9
	18	56	6·81	33	11	9	14	10	8	47	9
	18	92	1·24	26	15	11	23	14	12	49	11
Pill-boxes:											
Fed daily on rat	20	92	1·40	20	32	3	21	182	3	41	3
Fed once on rat	20	92	1·40	20	12	2	18	18	3	38	3
Not fed	20	92	1·40	20	9	2	24	14	4	44	3

humidity. The data show that the fleas fed daily lived longer than any others. The longevity of females increased with rise in relative humidity and corresponding decrease in saturation deficiency.

Specimens fed only once had the greatest maximum longevities when fed on a squirrel, rather than on a guinea-pig or on the natural host, *Microtus*. Under such conditions of starvation the effect of increasing humidity on longevity was slight.

Of the unfed fleas, those kept in pill-boxes did not live quite as long as those kept in glass tubes at corresponding temperatures and humidities. Again, the effect of increasing relative humidity was slight, but the longest-surviving fleas were those kept at the highest relative humidity. Also, the greatest median survivals were in the group kept at the highest relative humidity. A single individual, a female fed daily, survived for 6 months in a pill-box. The specimens kept in pill-boxes and fed once or starved had similar median longevities, but the maximum longevity was slightly longer in both sexes among the fleas that had a single blood meal.

The fleas given one opportunity to feed lived slightly longer than those not fed at all.

When the fleas were kept in pill-boxes and given daily opportunity to feed on a rat, maximum longevity was considerably extended for both sexes.

Table 9. *Data on the longevity of* Orchopeas sexdentatus sexdentatus

Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	Males: No. of fleas	Males: Maximum survival (days)	Males: Median survival (days)	Females: No. of fleas	Females: Maximum survival (days)	Females: Median survival (days)	Total: No. of fleas	Total: Median survival (days)
Glass tubes:											
Fed daily on guinea-pig	20	15	13·90	17	10	5	34	11	7	51	5
	20	56	7·72	28	12	5	21	8	5	49	5
	20	92	1·40	55	12	5	52	12	6	107	6
	13	15	9·55	17	6	4	30	6	4	47	4
	13	65	3·93	22	8	6	31	9	5	53	5
	13	94	0·67	19	7	3	31	6	3	50	3
Fed once on guinea-pig	20	15	13·90	15	8	6	22	11	6	37	6
	20	56	7·72	15	10	8	34	11	7	49	7
	20	92	1·40	37	10	6	55	12	5	92	5
	13	15	9·55	23	6	4	26	13	3	49	3
	13	65	3·93	22	8	6	28	11	6	50	6
	13	94	0·67	21	9	1	33	15	3	54	2
Not fed	20	15	13·90	21	10	7	34	9	6	55	7
	20	56	7·72	25	10	7	32	10	7	57	7
	20	92	1·40	92	9	3	93	8	3	185	3
	13	15	9·55	28	8	5	36	7	3	64	5
	13	65	3·93	16	7	5	24	7	4	40	5
	13	94	0·67	23	14	3	22	11	2	45	2
Pill-boxes:											
Fed daily on rat	10	94	0·55	17	48	2	9	319	4	26	3

Orchopeas sexdentatus sexdentatus (Table 9)

This flea, when held in glass tubes and fed daily on a guinea-pig, did not survive longer than when it was fed only once or not fed. Generally, survival of the three groups was a little greater at 20° C. than at 13° C., but increasing moisture content of the air did not exert a readily discernible beneficial effect on the longevity. In fact, in general those fleas held at the higher humidities did not live quite as long as those held at the lower humidities.

Megabothris abantis (Table 10)

M. abantis was kept only in pill-boxes. The longest-lived flea of any in the entire study was a female of this species which lived for approximately 16 months. After 390 days this flea was fed irregularly. The group of fleas fed regularly contained long-living individuals among both sexes, and considering the lot they survived a little longer at 20° C. than at 10° C.

Fleas given one chance to feed survived little longer than those never fed. Among these two groups the longest-lived individual was a female fed once and held at 10° C. It lived for nearly 3 weeks.

DISCUSSION

It is probable that keeping fleas in a container with moist sand as the Russians did supplied a saturated atmosphere much more favourable for the flea than conditions of the experiments being reported. The lower temperatures at which those fleas were kept much of the year may also have prolonged their lives. Tiflov & Ioff (1932) studied different species of fleas from those used in these studies, and it is possible that the potential longevities of these fleas differ. Bacot (1914) used paper containers for

holding fleas; in the present studies fleas usually survived much longer in pill-boxes than in glass tubes. At times Bacot also kept sand in the containers for his fleas to burrow in, and at other times he placed a piece of moist filter-paper with the fleas each day.

Of the longevity data found in the literature, those of the Russian workers indicate surprisingly long maximum longevities for both fed and unfed fleas. Although the maximum survivals of the fleas reared by Bacot were less than those of Tiflov & Ioff, his data also indicate that fleas maintained under favourable conditions have potentially long lives. The maximum longevities of *Xenopsylla cheopis* fed daily in the studies being reported exceeded that of the *X. cheopis* in Bacot's studies. The host in these studies was a rat; Bacot fed his specimens on man. For the unfed fleas, the maximum longevity of *X. cheopis* was about the same for both these studies and those of Bacot.

Nosopsyllus fasciatus, when fed daily on a rat, survived considerably longer than did the specimens in Bacot's experiments, but when kept unfed those of Bacot were considerably more long-lived. Bacot stated that *Pulex irritans* possessed a greater potential longevity than did the rodent fleas, and in his studies this flea survived longer than any specimen of the other species. The maximum longevities reported herein are:

	Maximum length of life in days by sex		
	Unfed	Fed once	Fed daily
Xenopsylla cheopis	40♂	41♂	158♀
Nosopsyllus fasciatus	13♂	22♂	281♀
Diamanus montanus	45♀	52♀	331♀
Oropsylla idahoensis	37♀	43♂	352♀
Opisodasys nesiotus	15♂	17♀	48♂
Malaraeus telchinum	15♂	20♀	182♀
Orchopeas sexdentatus sexdentatus	14♂	15♀	319♀
Megabothris abantis	10♂	19♀	477♀

The opportunity to obtain frequent blood meals had the greatest effect of any single factor in producing individual long-lived fleas.

Edney's work (1947) substantiated that of the Indian Plague Commission (1908), and of Mitzmain (1910) and others, that unfed fleas survived longer than fleas permitted to have a blood meal before starvation. Tiflov & Ioff (1932) obtained the opposite results, and maintained that fleas fed once survive longer than unfed fleas. These workers used different insects for their investigations, and it may be that what is true for rat fleas is not true for fleas of the sylvatic reservoir, or what is true for 'fur' fleas is not true for 'nest' fleas. In the studies being reported the longest-lived fleas, with the exception of *Opisodasys nesiotus*, were in all cases females that were maintained at relative humidities above 90% and fed daily. The experimental conditions selected were not favourable for *O. nesiotus* and probably more favourable conditions might have been obtained with experimentation.

Xenopsylla cheopis lived for about the same length of time whether or not they were given an opportunity to feed once before starvation.

For *Diamanus montanus* kept in glass tubes, the maximum longevity of unfed fleas and fleas given an opportunity to feed but once was attained by unfed fleas, and the median longevities were greater in this group also. However, of the fleas kept in pill-boxes the reverse was true.

All species studied had longer-lived individuals among those fleas which were fed once before being starved than among those which never were given an opportunity to feed.

Of all the fleas which were submitted to a period of starvation the longest-lived individuals of three of the eight species were males. In all instances but one, the longest-lived individuals of the starved fleas were kept at relative humidities over 90%.

Opisodasys nesiotus was not maintained under favourable conditions. It survived as well as some of the others when not fed or when fed only once,

Table 10. *Data on the longevity of* Megabothris abantis

				Males			Females			Total	
Holding and feeding	Temp. (° C.)	Relative humidity (%)	Saturation deficiency (mm. Hg)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Maximum survival (days)	Median survival (days)	No. of fleas	Median survival (days)
Pill boxes:											
Fed daily on rat	20	92	1·40	31	266	7	23	291	21	54	9
	10	94	0·55	32	11	4	27	477*	4	59	4
Fed once on rat	20	92	1·40	8	10	6	2	8	3	10	6
	10	94	0·55	13	5	4	16	19	6	29	4
Not fed	20	92	1·40	8	10	5	2	5	5	10	5
	10	94	0·55	24	5	3	21	6	3	45	3

* This flea was fed irregularly after 390 days.

but lived for a considerably shorter time than any of the others when given a daily opportunity to feed. This cannot be because it was unable to feed through the silk-bolting material, as its proboscis is structurally as favourable to such type of feeding as are the mouthparts of *Orchopeas sexdentatus sexdentatus* or *Megabothris abantis*.

All other fleas survived for approximately 6 months to a year or more. They could, therefore, survive sufficiently long to carry over plague infections during the interepizootic period.

Examination of the tables shows that pill-boxes or wood boxes are superior to glass tubes for holding fleas for extended periods. This may be due to a number of factors. Since the fleas kept in pill-boxes were fed by attaching the pill-boxes to the shaved abdomen of the host animal, it was not necessary to handle these fleas daily, as it was those fleas kept in glass tubes. Fewer specimens were kept together in the pill-boxes too, and this undoubtedly prolonged the lives of some fleas. Again they fed in the dark, while those fleas kept in glass tubes were offered opportunity to feed only in the light. The paper pill-box could come to equilibrium with the humidity at which it was held, and at the higher humidities this would beneficially affect the fleas during the time they were out of the humidity chamber for feeding on a host animal.

Cragg & Swaminath (1923) observed that *Xenopsylla astia* laid very few eggs if kept away from the host for the night. In these studies being reported, eggs were never found in any of the containers. This is further evidence that the conditions were not favourable for normal life of the fleas. Probably insufficient feedings and less sexual activity combined to prevent egg production among those fleas kept in containers and away from the host except for short intervals. It has frequently been observed that fleas copulate while on the host.

It is probable that some individual fleas, living under optimal conditions of temperature, humidity and host availability, in nature, live far longer than the longest-lived laboratory-reared and maintained fleas.

SUMMARY

The longevity of laboratory-reared fleas, two species parasitic on domestic rats and six species parasitic on wild rodents of the sylvatic plague reservoir, was studied. The 10,795 adult fleas used in these studies were maintained at different temperatures and humidities within a range encountered in their natural habitats. Different conditions of host availability were simulated by feeding some adult fleas only once, at the beginning of an experiment, by feeding others daily, and by not feeding others at all.

Generally, a single blood meal exerted a beneficial effect on the fleas in that those fed before starvation survived a little longer than those that were never fed.

With the exception of *Opisodasys nesiotus*, all fleas were potentially long-lived under some of the laboratory conditions. The longest-lived fleas in all instances were those kept at high humidities (90% relative humidity or more) and fed daily. An *O. nesiotus* male survived for 48 days, the longest period of any of this species. The maximum longevity for each of the other species was among females and was as follows: *Xenopsylla cheopis*, over 5 months; *Malaraeus telchinum*, over 6 months; *Nosopsyllus fasciatus*, over 9 months; *Orchopeas sexdentatus sexdentatus*, 10½ months; *Diamanus montanus*, over 11 months; *Oropsylla idahoensis*, nearly 12 months; *Megabothris abantis*, nearly 16 months.

The author wishes to express his appreciation of the technical assistance of Quentin Tomich during a part of these studies.

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COMPARATIVE STUDIES ON HOLOTRICHOUS CILIATES IN THE *COLPIDIUM-GLAUCOMA-LEUCOPHRYS-TETRAHYMENA* GROUP

II. MORPHOLOGY, LIFE CYCLES AND SYSTEMATIC STATUS OF STRAINS IN PURE CULTURE*

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(With 11 Figures in the Text)

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I. INTRODUCTION

In the last 30 years some fifty strains of holotrichous hymenostome ciliates, belonging to what has been designated the '*Colpidium-Glaucoma-Leucophrys-Tetrahymena* group', have been established in pure culture in various laboratories in the United States, France and England and have been employed in over 300 investigations principally of a physiological or biochemical nature. In very few of the publications is there evidence of any real attempt at first-hand identification of the experimental organism based on critical morphological and taxonomical study. Until about 1945 most of the ciliates being used were placed in at least four genera and in about twelve species; in that year, however, the influence of a careful investigation by Furgason (1940) was at last felt. Furgason studied critically representative species of the genera *Colpidium*, *Glaucoma* and *Leucophrys* and also several strains of a fourth species which had been established in pure culture and concluded that another genus was indicated for the latter. He proposed both a new genus and a new species, *Tetrahymena geleii*, based upon his comparative morphological studies and upon an extensive review of the literature. Since 1945, in America, nearly all small holotrichous ciliates which had or have been established in axenic culture (i.e. free from all other organisms) have been labelled, wholesale, as strains of *T. geleii*; indeed, the specific name has even been applied to extinct strains whose exact morphology can never be determined. In Europe, on the other hand, the name *Glaucoma piriformis*, stemming principally from Maupas's (1883) classical work, has continued

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to prevail as the most popular designation for what are probably very closely related ciliates. Recently in France the name *Leucophrys piriformis* has been proposed for several of the strains (cf. Fauré-Fremiet, 1949; Lwoff, 1950; and especially Lwoff, 1951, in footnote, p. 325). Possibly in many cases disposition of the strains to any one of the above-mentioned 'species' has been based upon unpublished comparative morphological studies; if so, the fact has been rarely indicated in the literature.

The concept of strain designations itself is an excellent one when it is known that the organisms to which they are applied are truly members of the same species. Furgason (1940) investigated only four strains, one now extinct, and he himself repeatedly suggested caution regarding the possibility of identity of the European *Glaucoma piriformis* of Dr André Lwoff and several other American pure strains which bore such names as *Glaucoma pyriformis, G. ficaria, Colpidium campylum,* and *Trichoda pura.* He stated: 'Whether they represent the same or different species we cannot say until they have been adequately studied...it is much to be desired that these several workers publish adequate morphological evidence of the nature of their strains...[whose] real identity must await careful morphological analysis.' And he added: 'Significant comparisons of experimental results cannot be made when the experimental animals represent the unknown factor.'

Of the twenty-nine pure culture strains in the *Colpidium-Glaucoma-Leucophrys-Tetrahymena* group (hereafter abbreviated to the C-G-L-T group) which are known to be still in existence in various laboratories in the United States and Europe, twenty-six have been obtained by the writer and a detailed comparative study of these ciliates forms the subject of the present paper. The three pure strains not included here have been considered as members of the species *Glaucoma scintillans* by the investigators who have used them. The majority of the remaining strains usually are considered to-day as members of the genus *Tetrahymena,* although evidence for such allocation generally has not been published. Furthermore, several strains are still carried as various species in other genera. The long and involved taxonomical history of all hymenostome ciliates in the C-G-L-T group which have ever been established in axenic culture has been traced in detail in a separate paper (Corliss, 1952*a*). Because of the necessity for proper orientation, an abbreviated history of the twenty-six strains under present consideration is offered in Table 1.

The ability of these protozoa to thrive in axenic culture on chemically analysable media has and is making them extremely valuable as the experimental animal in work already completed and in progress in several dozen laboratories, principally in the United States, England and France. Various of the strains have been employed to advantage in a wide range of investigations including studies on growth, nutrition, respiration, serology, morphogenesis, and facultative parasitism. Also being raised in pure cultures they have served as ideal food for certain other animals. Their recent adoption as microbiological assay organisms may prove to be of considerable significance. It is particularly unfortunate, therefore, that so much confusion has existed regarding the identity of the forms employed. In enthusiasm over their physiological potentialities workers for the most part seem to have neglected their morphology and taxonomy.

II. MATERIALS AND METHODS OF INVESTIGATION

Subcultures of the twenty-six strains of pure culture ciliates under present consideration were obtained through the kindness of American, English and French investigators whose names may be found listed on p. 84. Information regarding exactly which workers supplied what strains has been given in an introductory paper (Corliss, 1952*a*). Several permanent preparations of strains L-FF and FF-51 were loaned by Professor E. Fauré-Fremiet for inclusion in this study.

All strains, except FF-51 which never reached New York from Paris alive, were maintained at room temperature in cotton plugged test-tubes in two stock series: axenically in an autoclaved 1% peptone medium, and with unidentified bacteria in an autoclaved timothy hay (*Phleum pratense* L.) medium. Some of the strains were later cultured on the cleaned but not sterilized intestine of chironomid larvae (*Chironomus plumosus*) plus several drops of boiled tap water in plastic depression slides kept in a moist chamber. Most of the cytological work was based upon bacterized 'hay tea' cultures, since the ciliates in the C-G-L-T group are believed to be principally bacteria-feeders in nature. Transfers to fresh media were made regularly, using accepted bacteriological techniques, and routine tests for contamination of the axenic cultures were made. Living material was studied by standard methods, including use of hanging-drop preparations; phase microscopy was employed to a limited extent. All critical work was done under oil immersion.

Except for an additional series of nuclear stained material for which it was desired to have an abundance of young, actively dividing forms, most of the permanently stained preparations were made from cultures 5–7 days old. At this age, under the conditions noted above, these ciliates are usually still in the maximal stationary phase of growth, sizes and shapes are most uniform, dividing forms are infrequent, and maximum sizes are obtainable. For

Table 1. *Abbreviated history of pure culture strains**

Strain designation	Discoverer of strain and 1st publication†	Nomenclature formerly employed
	Tetrahymena pyriformis (Group 'A' species)	
GP	Hetherington (1934)	*Glaucoma pyriformis, G. piriformis, Tetrahymena geleii*
Ch-S	Chatton, in Chatton & Chatton (1927)	*Glaucoma* sp. *G. piriformis*
L-I	Loefer, in Corliss (1949)	*Tetrahymena geleii*
LR	Phelps, in Corliss (1950)	*T. geleii*
EZ	Phelps, in Corliss (1950)	*T. geleii*
HS	Phelps, in Corliss (1950)	*T. geleii*
L-II	Loefer, in Corliss (1949)	*T. geleii*
N	Phelps, in Corliss (1950)	*T. geleii*
GHH	Barber (1944)	*T. geleii*
T	Thomas, in Taylor *et al.* (1933)	*Colpidium campylum, Tetrahymena glaucomiformia, Glaucoma pyriformis, Tetrahymena geleii*
L-III	Loefer, in Corliss (1950)	*T. geleii*
Gf-J	Johnson (1935)	*Glaucoma ficaria*
GC	Glaser & Coria (1930)	*Trichoda pura, Tetrahymena geleii*
Y	Phelps, in Corliss (1950)	*Tetrahymena geleii*
W	Claff, in Kidder *et al.* (1940)	*Glaucoma ficaria, Tetrahymena geleii, T. piriformis*
GL	Lwoff (1923)	*Colpidium Colpoda, Glaucoma piriformis, Tetrahymena geleii, T. piriformis, Leucophrys piriformis*
T-P	Subculture of T; Phelps (1935)	*Glaucoma pyriformis, Tetrahymena glaucomiforma, T. geleii*
H	Hetherington (1932)	*Colpidium campylum, Glaucoma pyriformis, G. piriformis, Tetrahymena geleii*
E	Elliott (1933)	*Colpidium striatum, Glaucoma pyriformis, Tetrahymena geleii*
S	Seaman, in Wilber & Seaman (1947)	*Colpidium campylum, Tetrahymena geleii*
G1-R	Robertson, in Lawrie (1935)	*Glaucoma* sp., *G. pyriformis, G. piriformis*
	Tetrahymena vorax (Group 'B' species)	
PP	Barber, in Kidder & Dewey (1945)	*Tetrahymena vorax*
V_1	Lilly, in Kidder & Dewey (1945)	*T. vorax*
V_2	Lilly, in Claff (1947)	*T. vorax*
D‡	Lilly, in Kidder (1939)	*Glaucoma* sp., *G. vorax, Tetrahymena vorax, Leucophrys patula, L. vorax*
	Tetrahymena patula (Group 'C' species)	
L-FF	Lwoff, in Fauré-Fremiet (1948)	*L. patula*
	Tetrahymena faurei (Group 'D' species)	
FF-51	Fauré-Fremiet, in Corliss (1951*b*)	*Leucophrys* 51, *Tetrahymena* sp.

* Essentially a condensation, slightly modified, of Table I in Corliss (1952*a*)

† The investigator who established the ciliate in axenic culture (in general the same person who originally isolated it from nature) may not have been the first worker to publish on his strain. When such has been the case, the paper cited is preceded by 'in' although in some instances the name of the discoverer actually has not been given in that particular paper. Note also that the date of the first publication does not signify the year of isolation of the strain. Such additional details in the history of these protozoa have been given by Corliss (1952*a*).

‡ Now extinct, but included here because most of the references in the literature to *T. vorax* have been concerned with this particular strain.

the sake of uniformity all strains were handled by techniques as identical as possible.

The three main kinds of stains employed may be mentioned briefly. Of relief staining methods, China blue (=aniline blue) was found to give consistently the most satisfactory results. The body form is not appreciably distorted and, by focusing, the ciliary meridians can be observed all the way around the body; the cilia are nicely revealed and various openings through the pellicle are made clearly visible. For study of the nuclear complex, the Noland-Osterud stain was employed in temporary preparations. The standard Heidenhain iron-haematoxylin method, carried out in bulk using the centrifuge at every step, and the Feulgen reaction, done directly on the slide after initial fixation with corrosive acetic sublimate, were used for permanent preparations; only the Feulgen technique was considered sufficiently critical in the case of these small ciliates whose micronuclei regularly extrude or lose small chromatin-clumps which at times are difficult to differentiate from micronuclei and whose true micronuclei, in some strains, are permanently absent. The most extensive work was done on the various taxonomically valuable pellicular structures as revealed by silver nitrate techniques. Klein's dry method, using a monochromatic ultraviolet light (2537 A.) for reduction of the silver, was employed for the major part of the present investigation; later the Chatton-Lwoff impregnation technique, fixing the animals preliminarily with Champy and DaFano fluids and enrobing them in a thin layer of saline gelatine (cf. especially Chatton & Lwoff, 1936), reduction being carried out under a polychromatic lamp, was used to some extent in additional study of several strains.

Very early Klein (1926) pointed out the value of his silver technique for taxonomic work although he made little attempt to so use it himself. Chatton & Lwoff and their followers, although disagreeing strongly with Klein (and von Gelei) regarding the nature and function of the 'silver-line system' or 'argyrome', nevertheless have used their modification of Klein's technique to reveal the 'infraciliature', essentially the basal granules of the cilia, of a great variety of ciliates and have brought to light constant morphological features of considerable value in systematics. More recently Fauré-Fremiet and his students have applied silver impregnation, Chatton-Lwoff modification, in extensive studies on morphogenesis and comparative morphology in several large groups of ciliates with particular attention to the vestibular and buccal ciliature; there is already evidence that these last-mentioned investigations will contribute heavily in a taxonomic revision of the entire class Euciliata and of the holotrichous protozoa in particular (cf. Fauré-Fremiet, 1950). In the present critical work on such relatively undifferentiated ciliates as those in the C-G-L-T group the great advantage of silver methods lies in their unique revelation of anatomical features which often may be minute but which are consistently enough characteristic to be of significant comparative value.

III. COMPARISON OF STRAINS WITHIN FOUR GROUPS

(i) *Strains in group* 'A'

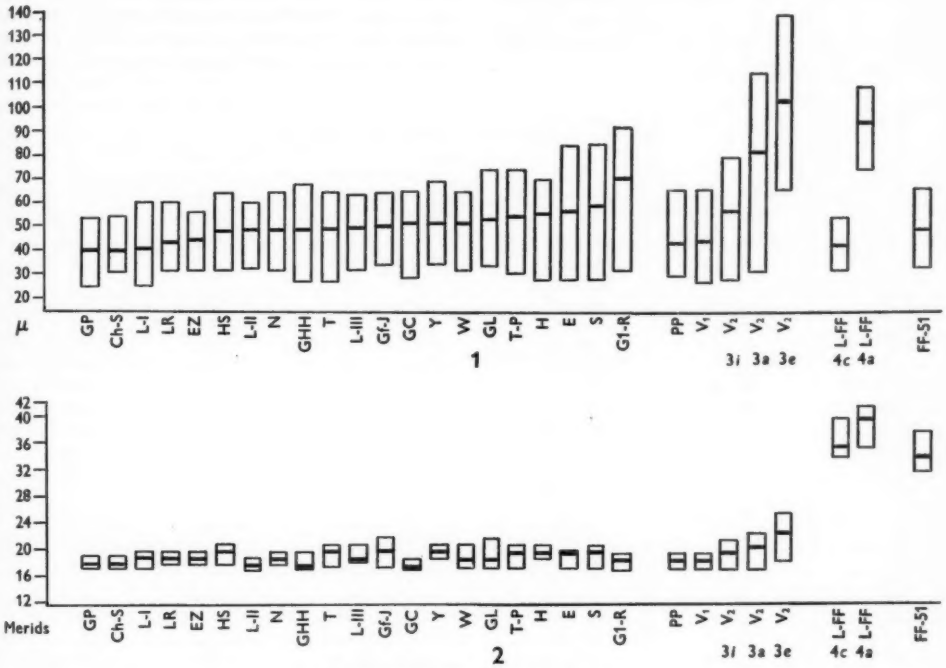
Study of similarities and differences among twenty-one of the twenty-six pure culture strains suggested such close relationship as to warrant consideration of these ciliates as members of a single group,* here designated as 'A'. These twenty-one strains possess in common: original isolation from fresh-water habitats; apparent inability to conjugate; similarity in general swimming movements; simple life histories; identical arrangement of component parts of their tetrahymenal buccal apparatus; and similarity in the majority of all other anatomical features, major and minor, which are revealed by relief staining, silver impregnation, and nuclear staining techniques.

Over 150 silver impregnated specimens of each strain, chosen at random from several slides, were used in taking measurements of various structures. Actually many hundreds more were observed and studied but it was considered unnecessary for statistical purposes to record data on more than 150 of them. Individual strain differences are included in Table 2; note that nowhere can a sharp line of demarcation be drawn as regards any of these characteristics. The order is based arbitrarily upon mean body lengths, the first strain being separated

* After this paper was in its final form Dr Muriel Robertson, Lister Institute, London, generously loaned me a letter, dated 7 December 1938, written by the late Dr Edouard Chatton in which Dr Chatton expressed his belief in the specific identity of six of the axenic strains included in the present work, five of which were employed by Robertson (1939*a*, *b*) in her serological investigations. Through the kindness of Professor E. Fauré-Fremiet I have been given access to the pertinent portion of the Chatton collection of protozoological slides and have been able to study his silver impregnation preparations, all dated September 1938, of the following strains (Chatton's own designations are given in parentheses): GP (H_1), Ch-S (C), Gf-J (H_2), GL (L), E (E) and G1-R (R). No significant differences from the data on the same strains as given in the present paper were found. Dr Chatton had labelled them all as members of the species *Glaucoma piriformis* and had intimated in his letter to Dr Robertson that he was planning to publish his investigations on them soon. Apparently the approach of World War II disrupted his plans at that time and his conclusions have never appeared in print.

from the last by a quite evenly graded series of intermediate lengths (cf. also Figs. 1, 2. That all of these ciliates are capable of great variation in body size, dependent upon environmental conditions, is given represent the typical appearances of entire populations at the time sample specimens were taken from the cultures.

In regard to the above discussion, a paper recently



EXPLANATION OF FIGS. 1–11

All the figures of ciliates are semi-diagrammatic, prepared in general from preliminary camera lucida drawings of fixed material treated principally by the silver impregnation methods of Klein or Chatton-Lwoff. The figures are typical of representative stages in the life cycles of the organisms, specimens possessing average body size, mean number of ciliary meridians, and the like, being purposely chosen as models. All measurements were made with a calibrated micrometer in a × 10 Zeiss ocular, using a × 95 Spencer oil immersion objective.

Figs. 1, 2. Comparison of the twenty-six pure culture C-G-L-T strains included in the present study in regard to length of body and number of ciliary meridians. The data were obtained from measurements and counts made on over 150 specimens from each strain, ciliates having been cultured and fixed under standardized conditions. Inscriptions appearing under strain designations V_2 and L-FF—3*i*, 3*a*, 3*e*; 4*c*, 4*a*—refer to figures in Figs. 3 and 4 respectively. Strains of species 'A', *Tetrahymena pyriformis*, include GP through G1-R; of species 'B', *T. vorax*, PP through V_2, data being given for various stages in the life cycle of the last strain; of species 'C', *T. patula*, two forms in the cycle of strain L-FF; of species 'D', *T. faurei*, strain FF-51. Fig. 1. Comparison of body lengths. Top and bottom of each bar indicate, respectively, maximum and minimum figures obtained; heavy cross-line marks the mean value. Fig. 2. Comparison of number of meridians. Top and bottom of each bar indicate the range in total number; heavy cross-line marks the mode.

not to be denied and, ordinarily, use of body length as a taxonomic criterion may be considered as inadvisable. But it should be stressed that in the present work all the strains were cultured and handled by the same techniques and the figures published (Loefer, 1952), the manuscript of which Dr Loefer has kindly sent me, is of pertinent interest. Working under rigid controls, with axenic cultures of fourteen strains belonging to group 'A', Loefer has found that in samples fixed at 48 hr. of age these

strains fall into three size groups: (i) averaging less than 51 μ in length—GP, LR, HS, Y; (ii) from 55–65 μ—L-I, L-II, N; (iii) from 65–80 μ—EZ, L-III, W, T-P, H, E, S. Generally averages were computed from measurements made under × 440 magnification on twenty specimens fixed in Bouin's solution. For the most part these findings support the order of strains determined in the present work using older, bacterized animals.

Elliott (1935), in a preliminary morphological investigation, suggested that strains now known as GP, H, and E should be separated into three different species in two genera, H and E appearing more closely related to each other than to GP. From a statement made by Furgason (1940), one must assume that Elliott later considered any differences he had observed as being of only strain value. Note in Table 2 that H and E are both some distance from

Table 2. *Comparison of strains in group* 'A'

Strain designation	Range in body length (μ)	Mean length × width (μ)	No. of meridians		Mean mouth length (μ)	Distance of mouth from apex (μ)
			Range	Mode		
GP	26–53	41 × 29	17–19	17–18	10·0	4·5–5·5
Ch-S	31–54	41 × 28	17–19	17–18	10·0	4·5–5·5
L-I	26–60	42 × 29	17–20	19	9·5	4·5–5·5
LR	31–60	44 × 26	18–20	19	9·5	4·5–5·5
EZ	32–58	45 × 26	18–20	19	9·5	4·5–5·5
HS	32–64	48 × 36	18–21	20	10·0	5·5–7·5
L-II	33–60	49 × 32	17–19	18	9·5	4·5–5·5
N	32–64	49 × 29	18–20	20	9·5	4·5–5·5
GHH	28–68	49 × 31	17–20	18	10·0	4·5–5·5
T	28–64	50 × 30	18–21	20	10·5	5·5–7·5
L-III	32–63	51 × 31	19–21	19	10·0	4·5–5·5
Gf-J	35–64	52 × 31	18–22	20	10·5	5·5–7·5
GC	30–65	53 × 33	18–19	18	9·5	4·5–5·5
Y	35–70	53 × 37	19–21	20	9·5	4·5–5·5
W	32–65	53 × 34	18–21	19–20	11·0	4·5–5·5
GL	34–74	54 × 31	18–22	19–20	11·0	5·5–7·5
T-P	31–74	56 × 34	18–21	19–20	10·5	5·5–7·5
H	29–71	57 × 35	19–21	20	10·5	4·5–5·5
E	29–86	58 × 34	18–20	20	11·5	5·5–7·5
S	29–87	59 × 34	18–21	19–20	11·5	5·5–7·5
Gl-R	32–92	72 × 35	17–20	18–19	11·0	4·5–5·5

The body form of strains GP and Ch-S, under cultural conditions as described in Part II, is frequently not of the typical pyriform shape shown by the other strains. The entire body is shorter, the width considerably greater than one-half the length, sides well rounded out, posterior end fully hemispherical. Regarding this unusual ratio of width to length, data on strain GP given by Loefer (1952) reveal the same condition although attention is not called to it in that paper. Occasionally other strains assume this shape, a form here designated as 'GP-form'. Length of the cytostome is a very conservative feature and the range of its length within members of a single strain is extremely slight when compared with the range in body size. For example, two Gl-R specimens with body lengths of 32 and 92 μ may have corresponding mouth lengths of 9 and 12 μ.

That real strain differences do exist among at least several members of group 'A' has been attested to in a number of previous studies in the literature. GP and also recall the unique GP-form commonly assumed by members of the last-mentioned strain. Chatton & Brachon (1935) believed Ch-S represented a species separate from that containing Lwoff's GL strain, on the basis of minute variations in the composition of the ciliary meridians; in later papers Chatton and associates considered the two ciliates only as separate strains within a single species (for historical details cf. Corliss, 1952*a*). Note the separation of these two strains, Ch-S and GL, in Table 2.

Certain physiological differences which have been reported in the literature to date may be regarded as of differential value only below the species level; further consideration of such differences among various of the strains, scattered throughout the literature and often in need of confirmation by re-investigation, is beyond the scope of the present paper.

The serological evidence for varying degrees of interrelationship among a number of the strains is of considerable interest. The independent results of

three different groups of investigators appear to support the arrangement of the strains as it is given in the present work. Robertson (1939*a*), employing strains now known as GP, Gf-J, GL, E and G1-R, concluded that E and G1-R are so similar in their reactions that they might be considered examples of the same 'species' or even of the same 'serological variety', whereas GP, Gf-J and GL are 'serologically distinct' from each other and from E and G1-R. Inspection of Table 2 and Fig. 1 will reveal that the two last-mentioned strains are very close to each other and are at some distance from the other three which are scattered among the first sixteen strains. Tanzer (1941) using GP, H and E, concluded that these three are serologically distinct strains forming a closely-knit group and that H and E are somewhat more closely related to each other than to GP. Kidder, Stuart, McGann & Dewey (1945), studying antigenic relationships among strains GHH, T, W, T-P, H, E, PP and V_1 (the last two are members of group 'B'), concluded that among the first six strains quantitative differences of antigenic materials exist but that the last two, which 'appear to be nearly identical antigenically', are distinctly different from the other six strains.

(ii) *Strains in group* 'B'

In the majority of anatomical features, particularly in finer details, ciliates of the three strains in group 'B', PP, V_1 and V_2, resemble a typical member of group 'A'. Existing morphological differences may be correlated with the complex life cycle which the markedly polymorphic organisms of 'B' are, or have been, capable of undergoing. Characteristics affected during such a cycle include: shape of posterior end of body, body size, mouth size, distance of mouth from apex of body, total number of meridians, number of post-oral meridians, number and location of contractile vacuole pores. Strains PP and V_1 are nearly identical with each other in every morphological feature investigated; so little variation could be found in the study of several hundred specimens of these two strains that their data have been combined under the single heading of PP-V_1 in Tables 3 and 4. Their close relationship found in the serological work by Kidder *et al.* (1945), mentioned above, may be recalled.

Differences between PP-V_1 and strain V_2 appear to be correlated solely with the apparent loss of ability by PP-V_1 to undergo the transformations of a complex polymorphic life cycle; further discussion of such differences, therefore, will be reserved for Part V. Biochemically the three strains appear to be identical. Using the same tests which Kidder & Dewey (1945) employed in demonstrating differences between PP and V_1, found to be identical, and certain group 'A' strains, Dr D. M. Lilly (unpublished) has found that V_2 is similarly distinguishable from 'A' ciliates.

(iii) *Strains in groups* 'C' *and* 'D'

Since there is only a single strain in each of groups 'C' and 'D', no 'intra-group' comparisons can be made. Repeated attempts to locate identical ciliates in nature, with the plan of establishing them in axenic culture and then including them in the present study, were made on a number of occasions but were always without success.

IV. COMPARISON OF THE FOUR GROUPS OF STRAINS

Most of the significant morphological differences among strains of the four groups are included in Tables 3 and 4 and in Figs. 1 and 2. In the tables group 'A' is represented by its two extremes, strains GP and G1-R, and by a hypothetical generalized form, called Gen. 'A', whose figures are averages of all data for group 'A' strains. Group 'B' is represented by PP-V_1, consolidation of data on the two strains indicated, and by V_2-tail, V_2-pyrif., and V_2-mac., representing the principal stages in the life cycle of strain V_2. Group 'C' is represented by L-FF-mic. and L-FF-mac., forms at major stages in the strain's life history. Group 'D' is represented by its only strain, FF-51; the figures given are based on data made on forms fixed from laboratory-raised cultures, although the ciliate was discovered as a facultative parasite of an embryonic trout. For descriptions of the stages in the life cycles of V_2 and L-FF and for discussion of the differences and similarities between these two strains in such stages, see Part V below.

The important morphological similarities may be summarized as follows: all strains possess the same type of tetrahymenal buccal ciliary organelles and the orientation and shape of the buccal overture are, in general, identical; the typical body forms are very similar; the mode of stomatogenesis is identical among the strains of all four groups; the general arrangement and location of the contractile vacuole pores is the same although the number of the pores and the number of meridians involved vary; and also there exists very close similarity in the many minor structural details, such as the various cytostomal, meridional, and apical fibrils, which are revealed clearly only by silver impregnation techniques. In addition, the location, shape, and overall appearance of the macronucleus are very similar among all the strains.

On the basis of such close similarity in fundamental morphological characteristics, the members of all twenty-six axenic strains of the C-G-L-T group which are included in the present investigation may be considered as congeneric ciliates.

Table 3. *Comparison of groups* 'A', 'B', 'C' and 'D'

Group and strain designations	Range in body length (μ)	Mean length × width (μ)	No. of meridians		Mouth: mean length × width (μ)	Distance of mouth from apex (μ)
			Range	Mode		
'A'						
GP	26–53	41 × 29	17–19	17–18	10 × 5	4·5–5·5
G1-R	32–92	72 × 35	17–20	18–19	11 × 6	4·5–5·5
Gen. 'A'	26–92	51 × 31	17–22	19–20	10·5 × 6	5·0–6·0
'B'						
PP-V_1	28–65	45 × 29	17–20	18–19	9 × 5	5·0–6·0
V_2-tail	31–115	82 × 40	18–23	21	11 × 5·5	4·0–8·0
V_2-pyrif.	29–80	58 × 30	18–22	19–20	11 × 6	5·0–6·0
V_2-mac.	67–140	103 × 69	19–26	21–23	29 × 23	1·0–6·0
'C'						
L-FF-mic.	32–55	45 × 28	34–40	36–38	10 × 5	4·5–5·5
L-FF-mac.	75–110	95 × 54	36–42	40	29 × 21	1·0–3·0
'D'						
FF-51	34–68	50 × 31	32–37	34–36	10·5 × 5	4·0–11·0

Table 4. *Further comparison of groups* 'A', 'B', 'C' and 'D'

Group and strain designations	No. of post-oral meridians	Data on the contractile vacuole pores					
		No.		Meridians involved		Average diameter (μ)	Distance from end of body (μ)
		Range	Mode	Range	Mode		
'A'							
GP	2	2	2	5–6	5–6	0·8	6–7
G1-R	2 (3 rare)	2	2	5–6	5–6	0·9	8–13
Gen. 'A'	2 (3 rare)	2–3	2	5–7	5–6	0·9	6–14
'B'							
PP-V_1	2	2	2	5–6	5–6	0·8	6–7
V_2-tail	2	2–6	2–3	5–9	5–8	0·9	av. 18
V_2-pyrif.	2	2	2	5–6	5–6	0·8	8–10
V_2-mac.	2–4 (usually 2)	2–6	5	5–10	6–9	1·5	av. 16
'C'							
L-FF-mic.	4–5 (usually 4)	2–4	2–3	9–12	9–11	0·9	5–8
L-FF-mac.	3–5 (usually 4)	2–6	4–5	10–15	10–12	1·5	9–11
'D'							
FF-51	2	2–3	2	8–11	8–9	0·8	5–8

V. LIFE CYCLES AND OCCURRENCE OF POLYMORPHISM

(i) *General considerations and terminology*

The ciliates under present consideration undergo alterations in form, more or less marked, during their ordinary cycle of life, changes which may be correlated with environmental conditions. Although it is known that many ciliates become enlarged, more rotund, and more sluggish in their movements in the presence of an abundant food supply and return to smaller, slimmer, more actively moving forms under fasting conditions, it has been realized only recently how great the extent of change may be in certain species. When different forms of the same organism are described as entirely separate species, then recognition of entire life cycles becomes of pertinent value from viewpoints of systematics, comparative morphology and evolutionary relationships.

Recent papers of particular importance on truly polymorphic ciliates include those by Kidder, Lilly & Claff (1940), who studied an extinct strain of group 'B', by Fauré-Fremiet (1944, 1945), and by Fauré-Fremiet & Mugard (1949). In the last

work it was concluded that the organism known as *Glaucoma sagitta* in the literature is very likely only the fast-moving form of the species *Espejoia mucicola*, a species in which such striking dimorphism had never before been recognized. As the ecology and natural life histories of ciliates become better known it is probable that a number of other similar discoveries will be made, and it will not be surprising if additional species in the C-G-L-T group are found to be involved.

Various types of polymorphism may be recognized but presentation of a scheme of classification of them is beyond the scope of the present paper. In regard to the minor changes apparent during the life cycles of such microphagous bacteria-feeders as the ciliates included in group 'A', I shall employ the qualified term *limited polymorphism*.* *Adaptive polymorphism* might apply to the form changes realized when a normally free-living organism enters some other animal as a facultative or accidental parasite, as in the case of the species represented by strain FF-51. Finally, the ciliates of groups 'B' and 'C' may be said to exhibit *pronounced polymorphism* but of a type which is not of regular reoccurrence in the life cycle; these free-living ciliates do not have a life history based on a non-variable pattern with ordered stages as do most of the obligate-parasitic protozoa.

In the descriptions of life cycles exhibited by members of the pure culture ciliates I shall use, when applicable, what is essentially the terminology employed by Mugard (1948), modified and condensed to a certain extent, as follows: (*a*) The *theront*—form shown at the 'hunting' stage; moves very actively as prey or other food is searched for. (*b*) The *trophont*—form shown at the feeding or 'engorging' stage. (*c*) The *tomont*—quiescent, usually greatly enlarged, often encysted form at the pre-division stage. (*d*) The *tomite*—small but active forms arising from the preeeding form by a variable number of fissions, which have usually taken place in rapid succession; these 'metamorphose' into the generally larger and even more active theronts. Environmental conditions can cause all sorts of modifications in the general scheme of the cycle with consolidation of stages, omission of forms, reversible transformations, and the like. In the rather complicated cases of groups 'B' and 'C', in which the ciliates may exhibit limited polymorphism superimposed upon a striking but irregularly occurring dimorphic condition, the additional terms *macrostome form* and *microstome form* will be employed for the two extreme stages when dimorphism is exhibited; these names refer, respectively, to the relatively huge and small sizes of the buccal opening in different stages in the cycle.

* The word 'polymorphism' is used in reference to more or less stable form changes and not for mere transient distortions of shape made possible by the plasticity of the pellicle. In the latter regard it may be of interest to point out that all the pure culture ciliates under present discussion, and several other congeneric species as well, possess a body which is flexible and pliable to a remarkably high degree.

(ii) *Life cycle of group* 'A' *ciliates*

The twenty-one strains in this group are bacteria-feeders in nature and exhibit only limited polymorphism, as defined above. Although the differences in size, shape and motility do not involve or are not accompanied by profound structural changes, nevertheless failure to recognize the range of variation under dissimilar conditions has no doubt been a source of much of the confusion in the past literature concerning the identification of these animals. Well-fed bacterized forms are generally pyriform in shape (Figs. 6*a*, *b*), starving forms are more cylindrical and may become extremely attenuated. Fission takes place only among the well-fed forms; the lean forms, however, seem to possess great tenacity for living and may persist in old cultures for many months. Among the latter may be found some very small individuals with relatively greater width. Observations on forms raised in axenic cultures reveal more or less this same pattern in the life cycle although the cylindrically shaped ciliates never seem to become so markedly attenuated.

If members of a strain are cultured in a drop of boiled water plus the cleaned intestine of a chironomid larva the ciliates, now facultatively histophagic, undergo a life cycle in which the forms may be defined in the terminology given above. If the inoculum has been taken from an old bacterized culture of starving forms, these lean individuals are the theronts. The trophont stage is realized in a very short time, as soon as the food is discovered. After some 15–20 hr. of feeding, the ciliates, relatively large, rounded forms packed with refringent food masses, often retire from the intestine and settle down on the bottom of the container as tomonts. One typical binary fission occurs without formation of any cystic membrane; the two small but pyriform shaped tomites swim about, generally returning to the food directly as trophonts; the now abbreviated cycle is repeated as long as environmental conditions remain favourable for it.

(iii) *Life cycle of group* 'B' *ciliates*

Kidder *et al.* (1940), in work on strain D, a group 'B' ciliate now extinct, briefly described changes in body form correlated with experimental changes in diet. Such terms as 'life cycle' and 'polymorphism' were not employed and no references were made to classical investigations which have been made on

the life history of the closely related species known in the literature as *Leucophrys patula* (see group 'C' below). The following descriptions of the pronounced polymorphism exhibited by group 'B' organisms are based upon observations made upon living material and upon China blue and silver impregnation preparations, using principally strain V_2 ciliates from both pure and bacterized cultures. Some of the statistical data accumulated for various of the stages appears in Tables 3 and 4. Major forms in the life history are given semi-diagrammatically in Fig. 3.

Tailed microstome stage (Figs. 3*a*, 7*a*, *b*). The 'thin, tailed organism' reported as the principal form found in nature also predominated, for many generations, in the pure and bacterized stock cultures of strain V_2. It is a microstome form, i.e. the buccal opening is small, and except for the elongate posterior end, slightly larger overall body size, and greater range in number of meridians and of contractile vacuole pores, it is practically identical with the ciliates of group 'A'. There are several common tail types, in hay cultures, ranging from a long 'skillet-handle' to a very short and sharply pointed kind; in pure cultures the forms are more conservative. Superficially the living organism at this stage resembles Stokes' 'microscopic shark', *Dallasia frontata*. As a theront it seems to be satisfied, in general, with discovery of bacteria as food; its form changes but little as a trophont. It becomes broader with less pronounced tail process as a tomont. The tomites, arising by ordinary binary fission without cyst formation, immediately become theront-trophonts.

An interesting morphological type (Figs. 3*g*, *h*) occurs in this stage when the ciliates become starved. These forms, which may persist for many months, in hay cultures, are theronts to an exaggerated degree. They are commonly very elongate with long and sharply pointed tails, but the buccal opening is of the typical microstome form type and other morphological features also are identical with those found in forms of the normal tailed microstome stage. These very attenuated individuals revert to more typical shapes shortly after transfer to fresh medium.

Pyriform microstome stage (Figs. 3*i*, *j*). This is a curious stage in that forms in it are morphologically indistinguishable from typical group 'A' ciliates; it therefore warrants considerable discussion. The appearance of tailless, pyriform and cylindrically shaped individuals seems to be correlated with the length of time the cultures have been maintained as pure lines in the laboratory and also possibly with the kind of medium in which they have been grown. Most interesting, and perplexing, is the fact that the stage appears to be an irreversible one; i.e. when all the individuals in a culture have reached it they are totally incapable* of returning to a tailed form or of undergoing transformation to the macrostome form, a phenomenon to be described presently.

Strains PP and V_1 both were in this stage (cf. Fig. 3*j*) when axenic subcultures were obtained from several different sources in 1948, 1949 and 1950, although they had been isolated from nature in 1942 and 1940, respectively, as tailed microstome forms capable of transformation to macrostome forms. They have remained irreversibly pyriform to cucumber-shaped, microstome, tailless organisms even when subjected to a variety of environments including ones closely simulating conditions in nature, such as when being placed in mixed cultures with other protozoa. There is no published information regarding the dates when the ciliates of these two strains came to resemble group 'A' forms so indistinguishably; however, Dr D. M. Lilly (personal communication) believes that early in 1945 strain V_1 could still show striking transformations in form whereas PP had already lost the ability by that time.

Strain V_2, isolated from nature in 1947, was still capable of undergoing pronounced transformations of form when a pure subculture of it was obtained

* Under the environmental conditions to which they have been subjected in the present investigation, this is true. The problem should be investigated further since both the physiological and genetical aspects may be matters of considerable significance.

Figs. 3*a–j*. Forms in the life cycle of *Tetrahymena vorax*. Ciliates from strain V_2 were used for all figures except 3*j* which is based upon strains V_1 and PP. Detailed anatomical structures, such as the buccal ciliary apparatus and secondary meridians, have been omitted. Outlines of pharyngeal pouch and of food vacuoles are shown in dotted lines. Arrows indicate the usual sequence of form changes in the life cycle. (See text, Part V, for details.) Fig. 3*a*. Tailed microstome form. Figs. 3*b–d*. Transitional forms. Drawn from specimens from a 'zweigliedrige Kultur' containing strain W as prey organism. Note striking resemblance of form in Fig. 3*d* to *T. patula* in Fig. 4*a*. Fig. 3*e*. Mature macrostome form. Fig. 3*f*. Preceding form undergoing binary fission. Figs. 3*g*, *h*. Attenuated forms from starved cultures. Fig. 3*i*. Tailless microstome form eventually assumed by all the ciliates in my bacterized cultures of V_2. An apparently irreversible stage. Fig. 3*j*. Pyriform microstome form completely indistinguishable, morphologically, from typical members of the species *T. pyriformis*. Both axenic and bacterized stock cultures of strains V_1 and PP exhibit only this form, apparently an irreversible stage. Question mark over the arrow indicates that V_1 and PP probably passed through the Fig. 3*i* stage, since at one time they were able to exhibit all the other forms in the life cycle, and that strain V_2 conceivably may assume the Fig. 3*j* form in time.

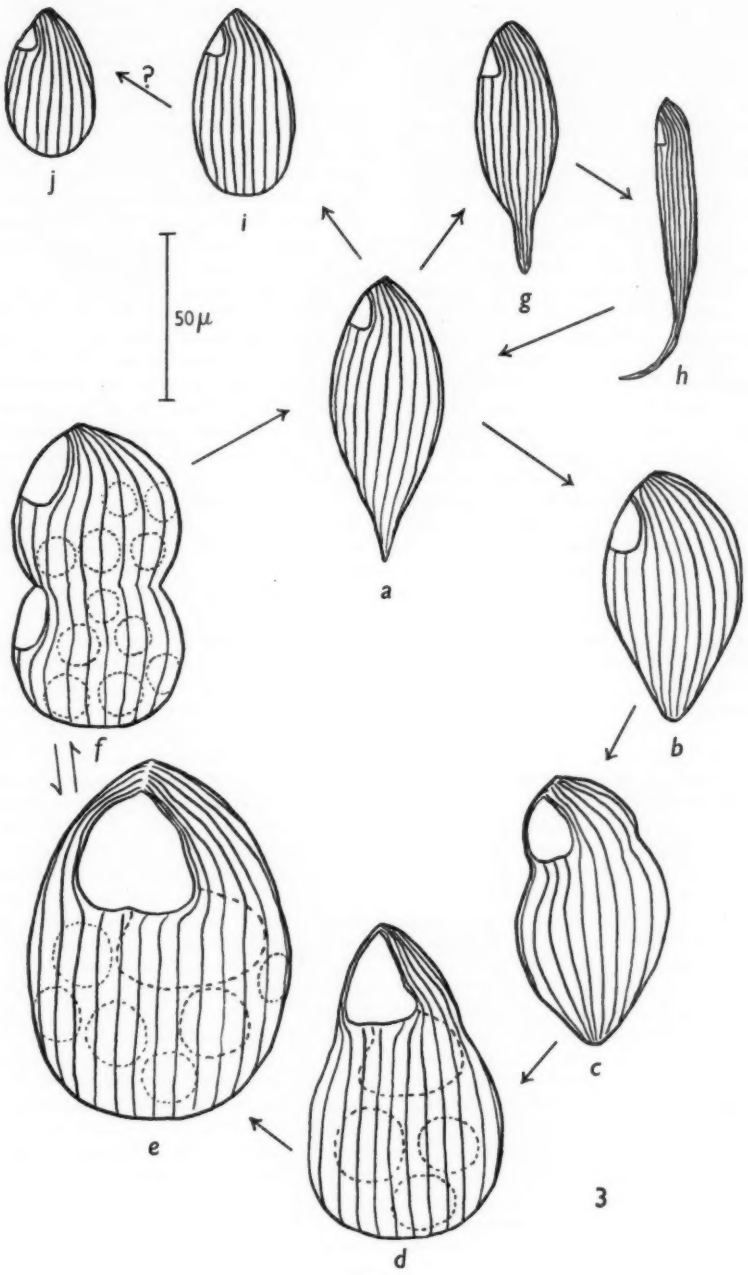


Fig. 3.

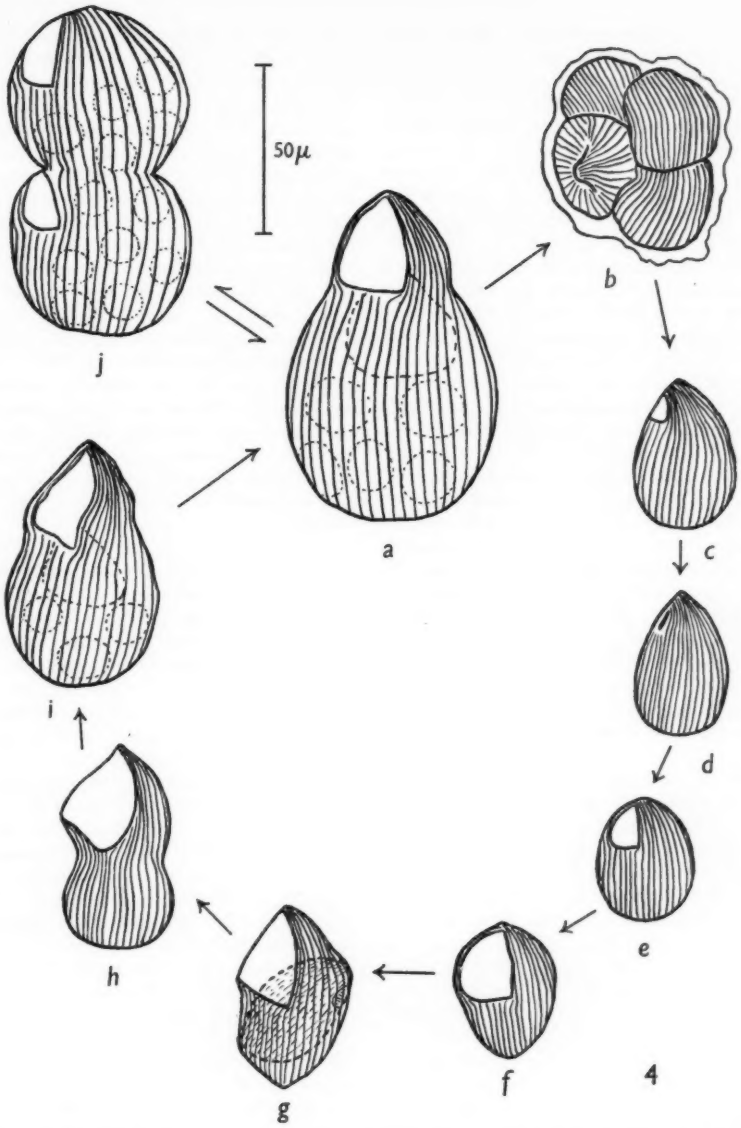
in December 1949. By August 1950 the organisms in my bacterized stock cultures had all become tailless (cf. Fig. 3*i*), very closely resembling group 'A' ciliates except for an average size slightly larger than that of the majority of 'A' strains. By that time they had thus entered the seemingly irreversible pyriform microstome stage. They persisted in this form, unchanged, no matter how they were treated, until the cultures were discarded in September 1951. On the other hand, the ciliates of my axenic stock cultures, which I am still maintaining, continue to remain in a typical bluntly tailed form and are still capable of transformation into the enlarged, macrostome form. This is indicated by the constant presence, in a fraction of 1 % of the population, of cannibalistic macrostome forms in the culture tubes; the number of these forms, which actually do not seem very voracious and contain but few large food vacuoles, always increases for a short time following regular transfer to fresh medium. And it is also demonstrable when a 'zweigliedrige Kultur' is established, either in pure or bacterized cultures, with animals from the axenic stocks and members of some group 'A' strain; non-cannibalistic carnivores appear in abundance within several hours.

Transitional stages from tailed microstome to macrostome form (Figs. 3*b*, *c*). Under conditions which in nature have not yet been studied but which may be realized experimentally in the laboratory, individuals in the tailed microstome stage can undergo transformation into enlarged, tailless macrostome forms. This has been observed for all members of group 'B' including strain D, now extinct; as pointed out above, however, only V_2 has been able to exhibit the phenomenon in recent years, under conditions employed.

When members of one of the strains in group 'A' are transferred as prey to either a pure or bacterized culture of V_2, the morphology of the latter is markedly and rapidly changed, resulting in a mature macrostome form in a very few minutes. A technique was developed for obtaining great numbers of transforming individuals in a small volume of culture fluid and it is upon study of such favourable material, both living and fixed, that the following condensed account is based. A broadening across the anterior third of the animal's body occurs first in the transformation; then the tail commences to fill out, eventually becoming non-existent as such. The buccal region of the body undergoes the most profound changes. The three membranelles of the left-hand adoral zone are pushed anteriorly, resulting in the obliteration of the pre-oral suture as such; and the striated portion of the undulating membrane complex is so enlarged that the rods or bars, as they appear in silver impregnated specimens, are trebled in number. As the buccal cavity is lengthened it is also widened, in even greater proportion, and a broad, pocket-like extension moves to the left, encroaching upon the space formerly occupied by portions of certain ciliary meridians. A pharyngeal pouch, the 'preparatory vacuole' of Kidder *et al.* (1940), develops from the distal portion of the formerly inconspicuous cytopharynx. As the voracious, carnivorous, seemingly never satisfied theront-trophont draws in more and more of its prey its pellicle becomes stretched over the food vacuole-packed body and, by silver staining, it may be realized now that the ciliary meridians have been increasing in number and in composition as well (see details in Part VIII).

Mature macrostome stage (Figs. 3*d*, *e*). Two forms, differing but little, predominate among the fully developed macrostome theront-trophonts: a '*Tetrahymena patula* form' which, superficially at least, is indistinguishable from the macrostome form of strain L-FF (cf. Fig. 4*a*), and a rounder, broader form in which the buccal overture is sometimes wider than it is long. Perhaps the latter may be considered as a true trophont-tomont, its most actively swimming and hunting hours temporarily over. The buccal opening of a typical macrostome form of strain V_2 has been figured previously (Fig. 11 in Corliss, 1952*a*). Division of the tomont, as the macrostome form may be properly considered for a short time in its life, proceeds normally with approximately equal distribution of food vacuoles and various other cytoplasmic inclusions to the two resulting tomites, the proter and opisthe (cf. Fig. 3*f*). No cystic membrane is formed and the tomites immediately become macrostome theront-trophonts. This cycle is repeatable again and again under favourable conditions. The return to the smaller, tailed microstome form occurs gradually by a succession of divisions in an environment in which the supply of prey has become depleted, as may happen in the case of non-cannibalistic carnivores. Why the percentage of cannibalistic carnivores in cultures free from other ciliates remains very low and practically constant over a long period of time, with slight and only temporary increase following transfer to fresh medium, is not known.

The initial stimulating factors which are responsible for the transformation into macrostome forms have not yet been identified. Kidder *et al.* (1940) suggested that the stimulus appears to reside in living, actively moving ciliated prey and that 'it may be... of a mechanical nature'. More recently a preliminary investigation by Claff (1947) has cast some interesting light on the problem. Claff found that if V_2 ciliates are separated from strain W (of group 'A') ciliates by a cellophane membrane, cannibals are produced in large numbers on the V_2 side of the membrane. If distilled water is substituted for the W organisms, only one or two cannibals



Figs. 4*a–j*. Forms in the life cycle of *Tetrahymena patula*. (See text for details.) Figures are drawn to the same scale as used in Fig. 3 and certain structures have been similarly omitted. Fig. 4*a*. Normal macrostome form. Note its resemblance to *T. vorax* in Fig. 3*d*. Fig. 4*b*. Production of tomites within delicate cystic membrane after culture of preceding forms had been subjected to starvation conditions. (Cystic membrane not impregnated with silver, added diagrammatically from observations on living material.) Figs. 4*c*, *d*. Pyriform microstome forms. In Fig. 4*c* note resemblance of ciliate to one shown in Fig. 3*j*. Fig. 4*d* portrays the mouthless stage. Figs. 4*e–i*. Transitional forms. *Colpidium campylum* was introduced as prey organism into culture containing forms in the pyriform microstome stage. Note in Fig. 4*g* the size of the ingested ciliate which, in this particular case, may have been a fellow member of *T. patula* devoured by a greedy and cannibalistically inclined congener. Fig. 4*j*. Macrostome form undergoing binary fission, normal method of reproduction when food is present in sufficient quantities.

appear among the population of the V_2 ciliates. This worker concluded that some factor from the W culture, diffusible through the semi-permeable membrane, must stimulate the formation of the carnivores. Further investigation along these lines should be fruitful.

(iv) *Life cycle of group* 'C' *ciliates*

Members of strain L-FF appear to be identical with the holotrichous ciliate known in the literature as *Leucophrys patula*, a matter to be considered in more detail in Part VII. The first investigator to describe the dimorphic life history of this species was Maupas (1888). Prowazek (1909) confirmed Maupas's careful observations and added information regarding the nuclear components. Lepşi (1926) wrote of the existence of polymorphism in the species as an 'Entwicklungszyklus'; Furgason (1940) also made brief mention of the occurrence of two forms and stated that a more detailed study of the ciliate would be included in a later paper. Fauré-Fremiet (1948), using strain L-FF cultured on a species of *Colpidium* and employing modern techniques which included silver impregnation, briefly confirmed both Maupas's and Prowazek's works and added some details on the anatomy of the macrostome form; Fauré-Fremiet's paper is chiefly concerned with experimental work on the production of homopolar doublets and their significance in the general problem of morphogenetic regulation in ciliates.

The account which follows does not, in general, contradict any of the findings of past investigators, although some new facts are recorded, new terminology is employed, and the entire description is based upon statistical study of several hundred specimens. Also silver impregnated microstome forms have never before been described. My first study of the life cycle, on which are based the figures given in Fig. 4*a–j*, was made on fixed material loaned by Professor E. Fauré-Fremiet, living cultures not having been acquired until a later date. Very recently I have had an opportunity to experiment with the animals under various laboratory conditions, producing the major forms in the induced cycle and several others besides; all of these are described below. The latter work has not been extensive, however, and there are a number of problems associated with the transformations in form which can be resolved only by further investigation at a future time.

Normal macrostome stage (Figs. 4*a*, 8*a*, *b*). This is the usual form encountered in nature and it is also persistent in normal laboratory cultures. The organism is an active and voracious hunter searching out and preying upon smaller ciliates; it appears to combine hunting and feeding with no particular changes in shape and thus may be considered to be a theront-trophont. The overall anatomy of the body and cytostome is strikingly similar to that of the V_2 macrostome form described on p. 60, the principal difference being L-FF's possession of nearly twice as many ciliary meridians. A very prominent feature, as also was noted for V_2, is the large pharyngeal pouch which may occupy a considerable volume of the second quarter of the body. During binary fission this pouch disappears and food vacuoles and other cytoplasmic inclusions are distributed more or less equally to the two daughter organisms (cf. Fig. 4*j*). The tomites, also macrostome forms, are thus produced from a tomont indistinguishable from the theront-trophont and there is no cystic membrane formed at any time. This abbreviated cycle is repeatable indefinitely not only in relatively natural environments with other protozoa as prey but also when the strain is grown as a saprozoite in autoclaved, non-particulate media such as enriched peptone broths.

Very recently I have obtained an interesting variation from the normal macrostome form just described. In bacterized cultures in which the supply of prey organism (strain S of group 'A') had become depleted 24–36 hr. previously, a number of forms small in body but typically large in mouth were noted. Some of these curious animals, in a stabilized form since they persisted unchanged for days, measured only 40 μ in length; the body shape, however, was exactly like the normal macrostome form except that relatively more area in the anterior half was devoted to the buccal overture. The ciliates also possessed the typical pharyngeal pouch although it remained empty, i.e. apparently nothing larger than bacteria was ingested as food. Dr André Lwoff (personal communication) has obtained similar forms of this same strain from time to time in various pure culture media. Fauré-Fremiet (1949) observed their appearance in experimental cultures to which certain chloride salts had been added. It is very likely the occurrence of this form, in Dr M. G. Brown's cultures of Dr W. H. Furgason's now extinct strain of '*Leucophrys patula*', which led to the following incorrect statement in Kidder *et al.* (1940, p. 19): 'In *Leucophrys* the large receiving vacuole appears to be a permanent structure (M. G. Brown, unpublished observation) and when the food ciliates are exhausted from the medium there is no change in form but simply a diminution of size.'

Microstome stages (Figs. 4*b–d*). Macrostome forms which become starved or are transferred into distilled water become a different kind of tomont from that described above and, upon dedifferentiation of all buccal structures, undergo from two to

four rapid fissions within fragile, temporary cystic membranes (Fig. 4*b*); this results in the formation of a number of small tomites. The latter are tailless microstome forms resembling the ciliates of group 'A' and forms of the irreversible stage in group 'B' to a remarkable degree, the greater number of ciliary meridians appearing to serve as the major morphological difference. In hanging-drop preparations I have recently observed the occurrence of two divisions within the literally invisible cyst. For some minutes before emergence from the cyst, the mode of which must be studied further, the tomites are exceedingly active; the squirmings of the tightly packed animals are rather amusing to watch because there seems to be nothing confining the creatures to such a small space. Upon release from the thin-walled cyst the tomites often appear as cylindrically shaped forms with truncate posterior ends and they rapidly swim about like typical theronts; several hours later they become, without intervening fission, the typical pyriform-shaped individuals (Fig. 4*c*). Using certain media, including autoclaved rabbit brain, Lwoff (personal communication) has produced populations of microstome forms which have persisted in axenic culture for several months at a time. That binary fission occurs at this stage under such conditions is a likely possibility but I have not observed its occurrence among the pyriform microstome forms persisting in my bacterized hanging-drop preparations.

A curious microstome form may next be reached by the pyriform-shaped tomites if they continue to be kept in a culture medium devoid of nutritive material; I am not certain, however, if this is an obligatory next step in the life cycle. The body becomes somewhat further reduced in size, although retaining more or less recognizable pyriform shape, and the buccal overture becomes reduced to a very narrow slit (Fig. 4*d*). It is interesting to note that the four post-oral meridians, characteristic of the species, remain unipolar even when all buccal structures have disappeared and the number 1 or stomatogenous meridian retains, latently, its powers of reproducing such structures when the proper environmental conditions become available. Whether or not the forms which have occurred in Lwoff's axenic cultures (see preceding paragraph) are without mouths is not known.

According to Maupas (1888, 1889), microstome stages occur naturally and in nature may serve the double objective of safeguarding the species and making possible 'sexual fecundation'. Conjugation is believed to take place only between microstome members of diverse clones.

Transitional stages from microstome to macrostome forms (Figs. 4*e–i*). When to a culture in the microstome stages just described some other ciliate is introduced as prey, an unknown stimulating factor causes extensive reorganization of both buccal and body structures, a transformation in general not unlike that already outlined for group 'B' ciliates. In the group 'C' organism, however, the cytostomial field is apparently affected precociously; individuals undergoing transformation have been observed which possess large, gaping mouths greater than one-half the length of the entire body. Thus equipped, they become, as Maupas suggested, voracious infusorian hunters, and can ingest successfully even prey with as great or greater body length than their own, the pliable pellicle bulging to accommodate it. In illustration of this, a young carnivore 'caught in the act' when fixed in preparation for silver impregnation has been depicted in Fig. 4*g*. These forms are truly theronts although after the first 'mouthful', which is taken very soon, the organism is already also a trophont. In a short time the body, like the buccal overture, is elongated and broadened and the mature, normal macrostome stage is soon reached by all the ciliates in the culture, if the prey organism has been provided in sufficient abundance.

It is important to note here that the small-bodied, large-mouthed forms which occur in the transformation just described are not identical with the unusual small macrostome form discussed on p. 62. The former are transient individuals and, as inspection of the figures in Fig. 4 will show, their forms are not small replicas of the normal, large-sized macrostome animal. The latter, on the other hand, persist in certain cultures and have a body shape very similar to their large counterparts: these forms may represent a partially arrested stage in the usual transformation, the mouth and pharyngeal pouch having developed, and the body form as well, but with the normal body size never being realized.

(v) *Life cycle of group* 'D' *ciliates*

The single axenic strain in this group, FF-51, was unique among the C-G-L-T group ciliates included in the present paper in that it had been discovered as a facultative parasite in the spinal canal of an embryonic trout. Subcultures of the ciliate failed to survive shipment from Paris to New York and also, unfortunately, the culture was irretrievably lost in Paris some weeks after its establishment in an autoclaved, bacteria-free medium. The description of FF-51 offered here and in Part VIII are based principally upon study of permanent preparations including sections of the infected trout with many of the parasites *in situ*.*

As indicated above, information on the life

* In several parts of the present paper, originally written some time ago, this strain is considered as extant, its loss having occurred relatively recently.

history of this ciliate is primarily limited to fixed material from free-living forms in laboratory cultures. The extent of morphological variation between such forms and those shown by the ciliate as a parasite cannot be determined very completely. Study of stained sections of the host reveal that FF-51 apparently possessed a broadly pyriform to ovoid shape in that environment; the body size is considerably smaller than the average for laboratory-raised individuals and the cytoplasm is packed with large granules of an unknown nature. The only clear-cut evidence of an adaptive polymorphism, as defined in the introductory section of this part of the present paper, is in the body size. Bacterized cultures were maintained with ease on the cleaned intestine of chironomid larvae; in such an environment the ciliates showed a more or less limited polymorphism as typically described for the group 'A' strains. Two forms, however, seem to predominate in the permanent preparations at my disposal, preparations for which the exact ages of the ciliates are not known. One, the most frequently seen, shows a typical pyriform shape (cf. Fig. 9*a*, *b*) and probably represents the organism in its trophont-tomont stage; the other is a curiously modified pyriform shape in which the anterior third of the body has become elongate and attenuated, perhaps characteristic of individuals in the theront stage. In the second form, smaller in body size, the buccal opening is generally twice as far removed from the morphological apex of the body as it is in the first form. This is shown in Table 3 in the data on range in distance of mouth from apex.

Because FF-51 appears to be identical with a ciliate described by Kozloff (1946), as will be discussed in Part VII, it will not be out of order to mention briefly the life cycle of Kozloff's organism, although the latter was never established in axenic culture. Found as a parasite in the liver and associated organs of certain slugs, the ciliate exhibited a change in form when cultured in the laboratory; one may consider the differences as illustrative of adaptive polymorphism. Kozloff noted that organisms from the 'parasitic phase' in the slug were typically cucumber-shaped and apiculate at the anterior end; sampled individuals from the 'free-living phase' were typically pyriform, not apiculate, and on the average some 20% shorter in length although of the same width. He observed that food vacuoles were considerably fewer in number in the relatively leaner forms in the slug liver but that after liberation from the host these individuals would 'gorge themselves on fragments of cells and droplets of fat'. One might consider the common parasitic form as at a theront-trophont stage, with emphasis on the theront, in its life history as a facultative parasite, and the common culture form, as described by Kozloff, as at a theront-trophont stage, mostly trophont, in a typical limited polymorphic kind of free-living cycle.

VI. ADAPTABILITY TO FACULTATIVE PARASITISM

Of the pure culture strains included in the present study one, FF-51 of group 'D', was discovered as an accidental or facultative parasite and another, GL of group 'A', has been found to thrive as one when inoculated experimentally into a variety of forms. Strain FF-51 was found in large numbers in all parts of the central nervous system and in the musculature of the tail of an embryonic trout (*Salmo irideus*), only 15 mm. in length, which had suffered a caudal injury. Professor E. Fauré-Fremiet, discoverer of the case, believes that the wound undoubtedly served as portal of entry for the ciliate (personal communication). This interesting example of accidental parasitism recalls to mind the reports of Epstein (1926) and Shumway (1940) who discovered '*Glaucoma*' species in the nervous systems of embryonic fish, species *Abramis brama*, and of an *Ambystoma* larva, respectively.

Strain GL became successfully adapted (i.e. the ciliates lived and increased in number but their presence invariably caused the death of their hosts) in the body cavity of caterpillars of the wax moth, *Galleria mellonella*, as shown by Lwoff (1924) and in the haemocoel of both larval and adult stages of some fourteen different insect species, *Dixippus morosus* being used most extensively, as revealed in the large-scale investigation by Janda & Jírovec (1937). The latter workers also inoculated the protozoa of strain GL into the body cavities of annelids, molluscs, crustacea, fish and amphibia, and, in addition, exposed the animals, purposely wounded, to cultures of the ciliates; all these attempts, however, failed to result in successfully induced facultative parasitism.

Recently I have discovered a number of naturally occurring cases of parasitism in the larvae of *Chironomus plumosus* by organisms identical with the group 'A' species. In other, or sometimes the very same, larval forms of *C. plumosus* I have also found what is apparently the organism described briefly by Treillard & Lwoff (1924); it may be considered to belong to a species* different from but very closely related to the group 'A' species. These two species thrive in bacterized laboratory cultures; no attempt has been made to date to establish strains of them in axenic culture although the value of accomplishing this with the Treillard & Lwoff form, which is often found undergoing conjugation,

* I consider it to be identical with *Glaucoma parasiticum* Penard, 1922; very recently this species has been transferred to the genus *Tetrahymena* by Corliss (1952*b*).

is recognized. Further work on the morphology, taxonomy, and physiology of the second species is now in progress.

In the literature of the last 30 years there have been about twenty reports of accidental or facultative parasitism exhibited by ciliates which may belong in the same genus to which groups 'A', 'B', 'C' and 'D' will be assigned in Part VIII. Many of these have been listed or discussed by Lwoff (1932), Kirby (1941) and/or Mugard (1948) and need not be repeated here. It should be pointed out, however, that the viewpoint commonly held—that these reports probably all involve strains of a single species (the present group 'A' ciliate)—should not be accepted without considerable caution. There is now evidence which shows that that idea is probably quite false. By means of an extensive re-examination of the original texts and figures involved and in conjunction with my own recently completed studies on both axenic and bacterized strains of many C-G-L-T group species, I believe that I have found clues which allow one to identify the various ciliates in question as members of at least three, and probably four, different species, three of which appear to be definitely congeneric. Detailed consideration of this matter is reserved for a separate paper, now in preparation. One case, probably the most enigmatical, which, in the light of certain recent findings, particularly warrants careful reinvestigation, is the first report of parasitism for holotrichous ciliates of this general group made by Lamborn (1921) with more detailed description of the material by Keilin (1921). The controversy with regard to this case revolves around the identity and significance of the cysts which Keilin noted studding the cuticle of the host's body (larval *Aedes* [*Stegomyia*] *scutellaris*) and which that author believed were formed by the ciliate parasitizing the mosquito. Watson (1946) has reported resistant cysts for a bacterized strain of group 'A',* the species to which Keilin's *Lambornella stegomyiae* has usually been allocated; on the other hand, I have recently found another species,* congeneric with group 'A' forms, which very readily forms resistant cysts. Muspratt (1945, 1947) has described cuticular encystment of a '*Glaucoma pyriformis*' which seems to be identical with the Lamborn-Keilin ciliate. Based upon these various reports and upon my own findings, the latter to be described in a subsequent paper, it becomes evident that the mode of encystment and the nature of the cysts themselves are very different in the cases of *Lambornella stegomyiae*, as reported by Keilin and Muspratt, the group 'A' ciliate described by Watson, and the species which I have observed. Whether or not the three species involved are congeneric is a matter which must await discovery once again of the Lamborn-Keilin ciliate, in great enough abundance to allow thorough study, including use of such modern techniques as silver impregnation. The possibility still exists, at our present state of knowledge, that *L. stegomyiae* should remain in an entirely separate genus, as it was originally disposed of by Keilin.

From reports in the literature, both natural and experimental evidence may be seen to exist in demonstration of the adaptability of certain free-living C-G-L-T ciliates to flourish as facultative parasites in body systems or cavities of a wide variety of hosts. Although most often found in nature in the haemocoel of insect larvae, these protozoa have also been recorded from the gills of a gammarid, from egg masses and liver of certain molluscs, and from various vertebrate tissues including especially the central nervous system of fish and of an amphibian and the infraorbital sinuses and alimentary canal of a bird. Since the last-mentioned case involved a ciliate described as identical with group 'A' strains of the present study and, principally because of its recency, has not been listed in any previous reviews, it is worthy of brief notice here. Knight & McDougle (1944) examined eight white Plymouth Rock cockerels, 9 weeks of age, and found very large numbers of active ciliates in smears from the crop, duodenum, small intestine, caeca, and particularly the infraorbital sinuses and eyelids. Preliminary study suggested that the organism belonged to the species *Tetrahymena geleii* Furgason, 1940, and more detailed observations on cultured animals, obtained from a nearby stagnant pool and assumed to be identical with the parasitic forms, confirmed the allocation. Effects of the infection were linked with possible vitamin A depletion in the host.

* Through the kindness of Dr Cecil A. Hoare, F.R.S., Wellcome Laboratories of Tropical Medicine, London, I have very recently been able to obtain for study a subculture of the coprophilic ciliate called *Glaucoma piriformis* by Watson. It is identical with my group 'A' forms in all respects. Its history is worthy of brief mention: the organism was found in a sample of a diarrhoeic human stool in May, 1939; however, Watson (1946), employing rigorous tests under controlled conditions, showed clearly that the ciliate could not have been a parasite in the patient. Since 1939 it has been maintained by Dr Hoare as a pure line bacterized culture on Musgrave and Clegg's agar covered with boiled water.

* *Tetrahymena* [*Paraglaucoma*] *rostrata* (Kahl, 1926) Corliss, 1952.

VII. SYSTEMATIC STATUS OF STRAINS*

(i) *Nomenclatural problem of generic name*

On the basis of data which have been given in Parts III and IV, one may justifiably consider the twenty-six axenic strains in the C-G-L-T group as members of a single genus, although in the literature various of them have been included in at least four different genera.† *Colpidium* and *Glaucoma* appear to be valid names for genera which have been legitimately established; however, from Furgason's (1940) characterizations of their type species and from my own unpublished studies on various species in these genera, I believe the ciliates under present consideration cannot be allocated to either of them. The choice of a generic name, then, would appear to be principally* between *Leucophrys* and *Tetrahymena*, two genera which Furgason preferred to keep separate but which I believe should be amalgamated. Choice of the name for such a consolidated genus involves nomenclatural problems of some complexity. Some of them have been considered briefly in an abstract (Corliss, 1951*a*) and their full resolution will be presented in a later paper. High-lights of the situation are as follows: Examination of the literature reveals that Ehrenberg's (1830) genus *Leucophrys* was considered by him to be the same genus as O. F. Müller's (1780) *Leucophra*, with merely an emendation of the spelling. Ehrenberg's *L. patula*, however, is neither an originally included species of Müller's genus nor identical with any of the four species which were originally included, therefore it cannot be type of the genus, as Furgason and others have claimed it to be; furthermore, it cannot even be retained in the genus *Leucophrys* (= *Leucophra*) because, as far as I have been able to determine, it does not appear to be congeneric with any of the original species in the genus. Thus if we hold that the name *Leucophrys* is unavailable for the ciliates under present consideration, on a legal technicality, and if the International Commission on Zoological Nomenclature will agree to consideration of *Tetrahymena* as a *nomen conservandum* with respect to certain other names actually having chronological priority (cf. col. 1, n. †), than the proper generic name for the twenty-six strains of pure culture ciliates will be *Tetrahymena* Furgason, 1940. (The above conclusions, in regard to '*Leucophrys*', are in conformity with the International Rules of Zoological Nomenclature as they now stand; cf. especially Article 30, sect. II, parts *e* and *f*, as published by Schenk & McMasters, 1948.)

(ii) *Taxonomy of group* 'A'

Data on the characteristics of the twenty-one axenically cultured strains in this group provide evidence for consideration of the ciliates as members of a single species.† This is the species described by

* A subsequent paper in this series on comparative studies of ciliates in the C-G-L-T group, based upon investigations just recently completed, will consider in particular the morphology and taxonomy of a number of fairly closely related species which have not been established in axenic cultures. Therefore the present study will be limited primarily to the interrelationships of the pure culture strains. Fuller discussion of the problems posed by von Gelei's (1935) *Colpidium glaucomaeforme*, Schewiakoff's (1889, 1896) *Glaucoma pyriformis*, Penard's (1922) *Glaucoma parasiticum* and Kahl's (1926, 1930–5) *G. pyriformis*, in my opinion all members of the genus *Tetrahymena*, as well as consideration of true members of the genera *Colpidium* and *Glaucoma*, will be reserved for the forthcoming paper. An attempt there will also be made to resolve the particularly enigmatic problems presented by Kahl's three species *Glaucoma maupasi*, *Saprophilus oviformis* and *Paraglaucoma rostrata*.

† A fifth genus, *Trichoda* O. F. Müller, 1773, one which has become extinct upon several different occasions in the past, has also been involved. In this case, however, the problem may be dismissed as purely one of nomenclature, and its resolution will be suggested in a separate forthcoming paper dealing with several nomenclatural matters related to this entire group of ciliates. There are actually a number of additional generic names involved and it is now evident that several of these, relatively obscure though some of them may be, have priority over Furgason's (1940) more recently proposed but very widely accepted name *Tetrahymena*. For what are believed to be justifiable reasons, too involved to be discussed in the present paper, proper petition will be made to the International Commission on Zoological Nomenclature to consider suppression of certain earlier synonyms in favour of conservation of the name *Tetrahymena*. Names which definitely will be affected include *Leucophrydium* Roux, 1899, and *Paraglaucoma* Kahl, 1926; *Saprophilus* Stokes, 1887, on the other hand, appears to be a valid genus although some of its species may have to be transferred to other genera. *Paraglaucoma* Warren, 1932, falls as a homonym of *Paraglaucoma* Kahl, 1926. Discussion of the extent to which names proposed for various ciliates found as facultative parasites are involved (including *Lambornella* Keilin, 1921, *Leptoglena* Grassé & de Boissezon, 1929 and *Turchiniella* G. & de B., 1929), will be reserved for a separate later paper.

* Cf. preceding footnote regarding suggested disposal of certain less well known or less critically defined genera whose species, at least in certain cases, are recognizably congeneric with the pure culture ciliates under present discussion.

† Since completion of the study on the axenic cultures I have examined a considerable number of additional strains isolated from nature and from the body cavity of chironomid larvae and have found them all to fall within the specific limits set by the data obtained on the group 'A' forms. Also studied and likewise found to be identical are the coprophilic strain from London (cf. p. 65, col. 1, n. *) and the ciliate advertised in recent years as *Colpidium striatum* by the Carolina Biological Supply Company in the United States.

Furgason (1940) as *Tetrahymena geleii*; but, in my opinion, his organism is probably identical with the ciliate described as *Leucophrys pyriformis* by Ehrenberg (1830, 1838) and as *Glaucoma pyriformis* by Maupas (1883). Consideration of these cases will follow shortly.

Principally because of the somewhat inadequate descriptions of several hymenostome ciliates by Stokes (1885, 1886, 1888), a number of the axenic strains have been assigned to the species *Colpidium campylum* (Stokes, 1886) Bresslau, 1922, and one to the species *C. striatum* Stokes, 1886. I have very recently completed a study of a group of congeneric forms which I assign to several species in the genus *Colpidium*, including *C. campylum* and *C. striatum*; clearly recognizable generic characteristics are revealed in silver impregnation preparations of these forms and, in my opinion, they preclude consideration of any group 'A' ciliates as members of that genus. *C. truncatum* Stokes, 1885, also appears to be a valid *Colpidium* species. *C. putrinum* Stokes, 1886, on the other hand, may quite likely be identical with forms of the axenic group 'A'; the original description is very meagre, however, and since the trivial name '*putrinum*' is antedated by Ehrenberg's '*pyriformis*' it may be dismissed from further consideration.

The name *Trichoda pura* Ehrenberg, 1831, has also been used for certain group 'A' organisms. For disposition of the generic name, cf. p. 66, col. 1, n. †; the trivial name '*pura*' is antedated by '*pyriformis*'.

Several of the ciliates have been assigned to the species *Glaucoma ficaria* Kahl, 1926. Although I have not yet been able to locate this ciliate in nature for purposes of re-examination of its morphology, the descriptions given by Kahl (1926, 1930–5) appear to differentiate it clearly from the group 'A' species and therefore the name should not be considered a synonym of the species here under consideration. In particular, the rounded anterior end of the body and the orientation of the buccal overture at an angle to the main axis of the body would seem to exclude *G. ficaria* from the genus *Tetrahymena*; whether it should remain in the genus *Glaucoma* is debatable but need not concern us here.

The *Colpidium glaucomaeforme* of von Gelei (1935) was recognized by Furgason (1940), from his detailed study on group 'A' strains T, H, E, and one now extinct, as belonging to the genus *Tetrahymena*, but he considered it a species (*T. glaucomaeformia*, emended to *T. glaucomaeformis* by Kozloff, 1946) separate from his own *T. geleii* because it appeared that von Gelei's organism possessed a greater total number of meridians with more of them joining the pre-oral suture, a larger buccal opening slightly further removed from the apex of the body, and a micronucleus apparently always present although inconstant in position. Furgason agreed that if it should eventually be shown that either his or von Gelei's species is capable of sufficient variation to obliterate these differences, von Gelei's specific name would become the valid name for all the ciliates concerned. The present study of the 21 group 'A' strains supplies data on variation within the species which may be considered to decrease significantly the magnitude of most of the differences named by Furgason. Very recently I have obtained a culture of a ciliate which is probably identical with *T. glaucomaeformis*; results of study of this organism will be reported in a later publication. Whether von Gelei's species eventually stands or falls, the trivial name '*glaucomaeformis*' is antedated by '*pyriformis*' and thus the nomenclature of the group 'A' protozoa, as proposed in the present paper, will not be affected should it be considered advisable to expand the specific definition slightly in order to include both forms.

In Maupas's (1883) 'contribution to the morphological and anatomical study of infusorian ciliates' an organism called *Glaucoma pyriformis* was described with the attention to fine detail so characteristic of all his work. Among a number of names he listed in synonymy was *Leucophrys pyriformis* Ehrenberg, 1830. Maupas did not have at his disposal the modern techniques indispensable for proper interpretation of all the buccal organelles in such a small holotrichous ciliate; nevertheless, there is nothing in his text or figures which appears to contravene the specific characteristics which both Furgason (1940) and I have found for the group 'A' species. That Maupas transferred this species to the genus *Glaucoma* where, as Furgason has ably shown, it cannot be retained was a small but most unfortunate error since it has been the cause of much of the subsequent confusion in the history of ciliates in the entire C-G-L-T group. Later Maupas (1888) did recognize close similarities between his *G. pyriformis* and the organism he called *Leucophrys patula* and had this second investigation, which was on *L. patula*, been undertaken before the first, on *Glaucoma pyriformis*, it is conceivable that the smaller ciliate might well have been retained by Maupas (and thus by subsequent authors) as a member of the genus *Leucophrys*, as this genus was characterized by Ehrenberg.

Although Ehrenberg's (1830, 1831, 1838) descriptions and figures of his *Leucophrys pyriformis* leave something to be desired in the matter of fineness of detail, there is nothing in his presentations which cannot be reconciled with Maupas's observations in the later worker's re-description of the ciliate under the name of *Glaucoma pyriformis* and with Furgason's and my own on various strains of group 'A' ciliates. Furthermore, the fact that Ehrenberg (1838) definitely stated that there are 9–12 ciliary rows on one surface of the organism (and his figures

show about 8–10) and the additional fact that he never observed conjugation in his cultures lend weight in favour of acceptance of the specific identity of all these ciliates. Furgason (1940), in writing of the occurrence of microstome forms in the life cycle of *Tetrahymena patula* (species 'C' in the present paper) stated: 'It is quite likely that these small *Leucophrys* represent the forms which Ehrenberg (1830) described as a new species *L. pyriformis*....' Since I have found from 34–40 ciliary meridians (therefore about 17–20 on one surface) in such microstome forms, Furgason's conclusion is possibly unsound. Any decision as to what organism Ehrenberg was really describing must admittedly be an arbitrary one since re-examination of his cultures of the ciliate in question is naturally impossible; that it may have been identical with the group 'A' species is not an illogical conclusion to draw, however, and the facts available may be considered to support this contention.

By application of the Law of Priority (Article 25 of the International Rules of Zoological Nomenclature), all more recent names may be considered as subjective synonyms of Ehrenberg's originally proposed name for the ciliate. As discussed above, however, it appears that the valid generic vehicle for this organism cannot be *Leucophrys*; pending approval by the International Commission on Zoological Nomenclature (cf. p. 66, col. 1, n. †), the name *Tetrahymena* is here considered proper. Lwoff (1947), without description, figures, or discussion, and employing the spelling 'piriformis',* used what I have concluded is the correct specific name for the ciliate in question. The full name, therefore, is considered to be *Tetrahymena pyriformis* (Ehrenberg, 1830) Lwoff, 1947. It is the type species of the genus, having been so designated, although under a different name, by Furgason (1940). Lwoff's strain GL, cultured axenically for 30 years, may be considered as type strain of the species.

(iii) *Taxonomy of group* 'B'

The three pure culture strains in this group, plus an extinct strain D, are or have been so closely related as to be obviously members of the same species, if one accepts the apparent irreversibility of the pyriform microstome stage in the life cycle of these ciliates (see Part V) as an explanation of the present state of limited polymorphism exhibited by strains PP and V_1 in both pure and bacterized stock cultures and by strain V_2 in bacterized cultures. Strain D was originally described as a new species in the genus *Glaucoma*, *G. vorax* Kidder, Lilly & Claff, 1940; it was subsequently transferred by Kidder (1941), without re-description of any kind, to the genus *Tetrahymena*. Based upon my own detailed observations on strains PP, V_1 and V_2, I am in agreement at the present time that the proper and full specific name for these organisms should be *Tetrahymena vorax* (Kidder, Lilly & Claff, 1940) Kidder, 1941.

The identical morphological picture given by members of this species which no longer seem able to undergo the transformation microstome form⇌macrostome form, and by the ciliates of group 'A', is further emphasized by the fact that all extant group 'B' strains are amicronucleate, as are all group 'A' axenic strains examined to date (see Part IX). It appears that the two species, once members of species 'B' have assumed the pyriform shape, can be distinguished only by biochemical and serological tests (cf. Kidder & Dewey, 1945; and Kidder *et al.* 1945). The only published use of these critical methods of differentiation appeared some 7 years ago; it would be worthwhile knowing if such tests still hold true for these axenic strains.

The problem of whether group 'A' and 'B' ciliates belong to a single species becomes particularly perplexing when one admits the possibility of all 'A' strains having formerly possessed and later lost the power of transformation into a carnivorous macrostome form. The constant absence of a micronucleus in strains of both groups serves to increase the attractiveness of such an hypothesis. Perhaps the only way to cast light on this possibility would be to find a method of experimentally inducing 'A' ciliates (and PP and V_1 of group 'B' as well) to undergo the life cycle so characteristic of the second species when the latter has been freshly isolated from nature. Dr G. W. Kidder (personal communication) once noted changes in body form occurring in several specimens of strain W which were being cultured in an amino-acid medium deficient in tryptophane. In a hanging-drop preparation a small number of organisms became carnivorous at the expense of others in the drop. This exhibition of cannibalism, however, was not investigated further and it is not known if the ciliates were truly macrostome forms. I have occasionally noted 'giants' in various group 'A' cultures but the buccal structures of the ones carefully examined have always been of the typical microstome type, a size which, however, would not allow ingestion of fellow strain members as was observed by Kidder.

Should future work decide that the '*Glaucoma vorax*' of Kidder *et al.* and the '*Tetrahymena geleii*' of Furgason are members of a single species then the trivial name '*vorax*' would take precedence over '*geleii*', for the former was published earlier in the year 1940 than the latter. However, in the preceding section it has been proposed that *T. geleii* should fall as a synonym of *T. pyriformis*, and '*pyriformis*' antedates '*vorax*'. Thus *T. pyriformis* still would

* The controversy over the spelling of this trivial name has been presented previously (Corliss, 1952*a*).

stand and *Glaucoma vorax*, *Tetrahymena vorax* and *T. geleii* would be listed as subjective synonyms of it.

(iv) *Taxonomy of group* 'C'

The pure culture strain L-FF is unquestionably identical with the ciliate known in the literature as '*Leucophrys patula* Ehrbg.'* and it was so designated in the experimental work performed with cultures of it by Fauré-Fremiet (1948, 1949). The early taxonomic history of this species is considerably confused because of Ehrenberg's (1830, 1838) inclusion of two quite different ciliates under a single name, both seeming to him to be identical with the *Trichoda patula* of O. F. Müller (1786) which he was transferring to the genus *Leucophrys*. (That none of his *Leucophrys* species should have been placed in such a genus, a separate problem from the one under present consideration, has been discussed briefly in sect. (i) of Part VII.) In spite of some confusion persisting in the literature even into the early 1900's, the problem was neatly resolved by Stein (1860, 1867). This investigator called one of the two ciliates involved, a heterotrichous form, *Climacostomum patulum*, basing his action on the detailed description of Claparède & Lachmann (1858); for the other, a hymenostome holotrich, he reserved Ehrenberg's (1830) name *Leucophrys patula*. Maupas's (1888) extensive investigation leaves absolutely no doubt as to what creature is intended to bear this name, and the recent brief descriptions by Furgason (1940) and Fauré-Fremiet (1948) have confirmed the taxonomic conclusions of Maupas and of various other earlier workers, several of whom have been cited above.

Corliss (1951*a*, *b*) has considered L-FF as congeneric with the other axenically cultured ciliates under present consideration and has proposed that the generic vehicle be *Tetrahymena*. Therefore the full name of this ciliate may be written *Tetrahymena patula* (O. F. Müller, 1786) Corliss, 1951.

(v) *Taxonomy of group* 'D'

Strain FF-51, unfortunately now extinct, appears to have been identical with the ciliate described in careful detail by Kozloff (1946) as '*Tetrahymena limacis* (Warren, 1932) nov. comb.'. However, having found another ciliate which I believe more nearly fits the description given originally in Warren's (1932) work, I have proposed (Corliss, 1952*b*) that Kozloff's organism and strain FF-51 be included as members of a new species, named *Tetrahymena faurei* in honour of its discoverer, Professor E. Fauré-Fremiet.*

Warren's and Kozloff's animals both were discovered as parasites in visceral organs of the slug *Deroceras agreste*. A third worker (Reynolds, 1936) also described ciliates from the same species of slug, assigning them to the trichostome species *Colpoda steini*; the incompleteness of Reynold's description makes it impossible to know for certain that it was truly a trichostome, rather than a hymenostome, species with which he was dealing. The fact that these three ciliates were all found parasitizing the same species of host does not, however, indicate that they themselves must be identical. In this regard my own discovery of the simultaneous infection of certain chironomid larvae by two different species is of pertinent interest (see Part VI).

The most apparent morphological difference between Warren's organism and *Tetrahymena faurei* is in the total number of ciliary rows; another difference worthy of mention is based upon the fact that conjugation was never observed to occur in the case of Kozloff's ciliate. In the ciliate which I have found and which I believe is identical with Warren's species conjugation occurs in a manner strikingly similar to that described by Warren. Regarding the number of meridians Warren stated in his text (p. 5): 'In a full-grown individual there are about twenty-six meridional grooves converging to a central point at the rounded posterior end of the body.' In his Pl. I, figs. 2 and 8, which are posterior polar views, he shows twenty-four in the first, twenty-one or twenty-two in the second. In various other figures of lateral or dorsal views he depicts striations which, if assumed to roughly approximate a few less than half the total number, give a range of about 24–29 meridians. In his most detailed drawing, of the ventral surface (his Fig. 1), he shows about 17 lines on the one surface which is quite inconsistent with the numbers given in other figures and in the text and may serve to emphasize the fact that he was not particularly concerned with showing an exact number in his illustrations (or that some of his slugs were parasitized by both species?). Kozloff, using silver impregnation which allows an accurate count to be made easily on a large number of organisms, reported a range of 33–37 ciliary rows for his ciliate. Employing

* As Fauré-Fremiet (1948) noted, it is very likely this same ciliate which Roux (1899) unfortunately chose to describe under entirely new generic and specific names, *Leucophrydium putrinum*.

* In my opinion, Warren's *Paraglaucoma limacis* is identical with the *Glaucoma parasiticum* of Penard (1922) which, as a member of the genus *Tetrahymena*, becomes *T. parasitica*, with *Paraglaucoma limacis* falling as a synonym. Since a number of ciliates in the literature appear to be members of this species, the particular strain described by Warren will be referred to in the present paper merely as 'Warren's ciliate', in order to avoid confusion.

over 100 specimens, also impregnated with silver, I have found the range in strain FF-51 to be 32–37. In this matter of number of meridians, certain of Kozloff's remarks (p. 452) are of interest: 'It is quite possible that there is considerable variation in the number of ciliary rows in this species. I have studied a ciliate infecting the liver of the snail *Monadenia fidelis* (Gray) which has twenty-six to twenty-nine rows, but which is in all other respects identical with *Tetrahymena limacis* from *Deroceras agreste*.' It would be interesting to know if conjugation ever occurred in Kozloff's laboratory cultures of the organism from the snail; on the evidence available I am inclined to believe that that ciliate may have been identical with Warren's parasite whereas Kozloff's slug parasite may be considered to belong to the species *Tetrahymena faurei*.

The problem which confronts one here, and also elsewhere when dealing with ciliates in the C-G-L-T group, is where to draw the line in regard to specific boundaries with forms which possess many identical morphological characteristics. It is complicated, as far as numbers of ciliary rows are concerned, by the general disregard in the past literature for determining the exact number: published drawings showing widely varying numbers in side-by-side figures of the same organism give evidence of this. And when the number has been given in the text, it is often totally disregarded in the plates. To-day, however, with various relief staining methods and in particular silver impregnation techniques, protozoologists have at their disposal methods which allow exact determination of the number of meridians, for ciliates such as those under present consideration, to be made with ease. I have studied a number of ciliates in which the typical body shape and size, the arrangement of the buccal ciliature, the overall nature of the meridians, including the pattern of their convergence at both poles, and the general location of the contractile vacuole pores are practically identical; yet, based upon counts made on many individuals, these ciliates appear to fall into the following categories, with regard to total number of ciliary rows: 17–23, 17–26, 24–29, 28–32, 32–37, 34–42. In most cases, admittedly, there are other differences, more or less distinct, in additional morphological features or in physiological characteristics. But if the number of meridians, by which the forms can be readily differentiated, if enough specimens are employed, is considered a factor of little taxonomic significance, then one might conceivably lump these forms into a single species containing a wide variety of 'strains' (present 'strains' then becoming 'substrains'†). Kozloff (1946) considered forms possessing about twenty-six and thirty-five ciliary rows as members of a single species; Mugard (1948) intimated the possibility of uniting groups having about nineteen and thirty-five as mean numbers of meridians in her statement (p. 260), 'Or les figures de Kozloff indiquent nettement que ce Cilié est un *Glaucoma* [*Tetrahymena*] du type *piriformis*'. On the other hand, Furgason (1940) suggested separation of the two extremes (in my series, above) into two different genera; at the time of his work, however, most of the 'intermediate' forms were either entirely unknown or unrecognized as very closely related ciliates. More extended discussion of this difficult and controversial problem is beyond the scope of the present paper.

VIII. DESCRIPTION OF GROUPS AS SPECIES

Although members of the four species included in the present study have been described to a greater or lesser extent in the literature, under a variety of names, it is considered justifiable to offer a rather complete description of them here, in many instances significantly revised or extended. This is the first time that these ciliates have been recognized as members of a single genus. Standardization of equipment and of techniques of investigation, as well as inclusion of a variety of strains and use of statistical methods, has made possible a uniformity in the resulting data which may be considered of value in such a comparative study.

(i) *Species* 'A' (*Tetrahymena pyriformis*)

Habitat. Fresh water, ranging from stagnant pools, streams, and ponds to thermal springs (40° C.). One report, probably of this species, from salt marshes (Fauré-Fremiet, 1912). Found in soil samples (Sandon, 1927); also considered a coprophilic protozoon (Watson, 1946). At least some strains capable of thriving within bodies of various metazoa (see Part VI). Very widely distributed species in both America and Europe.

Food. Normally bacteria in nature; can be cultured axenically in variety of autoclaved, nonparticulate media.

Conjugation. Never observed to have occurred in either pure or bacterized cultures, including mixtures of various strains. There are no reports of it in the literature except in the paper by Treillard & Lwoff (1924) who found abundant evidence of conjugation in a '*Glaucoma piriformis*' parasitizing chironomid larvae; I have rediscovered this ciliate however, and believe it to be a member of a separate species (cf. Part VI).

Cyst formation. Never reported for pure culture strains. Watson (1946) described and figured cysts and the process of excystment in a strain of '*G. piriformis*' which I have obtained and found to be identical with the group 'A' ciliate (cf. p. 66, col. 2, n. †). The cysts were obtained simply by allowing a culture on agar in a Petri dish to dry slowly. Because of

the importance of this heretofore undescribed phase in the life cycle of this species, I have attempted to duplicate Watson's findings. In a preliminary investigation, using both pure and bacterized cultures of several group 'A' strains and the Watson-Hoare strain as a control, I have not yet been successful in producing cysts, even in the control. Instead, I have observed occurrence of the phenomenon of anabiosis, somewhat like that exhibited by *Balantiophorus minutus* (cf. Watson, 1944). When the surface moisture of agar plate cultures was allowed to become slowly (length of time ranging up to several weeks) and completely dried up, the ciliates, seemingly lifeless with even apparent cessation of contractile vacuole pulsation, appeared extremely flattened and irregular in outline. Upon addition of water the grotesquely shaped forms arose immediately from the agar surface and beating of cilia soon commenced; normal shapes were assumed after several minutes of swimming about. There was no evidence of cysts or of excystment. When agar cultures were desiccated until the agar was hard and brittle, no ciliates appeared after addition of water to the dishes. These observations seem to bear out ones I have made many times throughout earlier work with the ciliates: if the fluid in stock culture tubes or in moist chamber depression slides has been allowed to evaporate seemingly almost completely, in several cases in tubes untouched for 10–14 months, addition of water or fresh medium produces an immediately teeming population of freely swimming organisms; if the tubes or slides have become very dry, no 'rejuvenation' of the protozoa occurs when the fluid is replenished. In the limited work I have done to date in attempts to rediscover Watson's cyst-formation phenomenon I have apparently overlooked some environmental factor of importance; further experimentation is under way. My recent isolation of a strain of this species from relatively dry moss might possibly be considered as indirect evidence that resistant cysts, under proper conditions, can be formed.

Body form. Typically pyriform or slightly elongated pyriform shape, the latter more common in pure culture, with bluntly pointed anterior end and rounded posterior end; ovoid, cucumber and rod or cylindrical shapes also very common, particularly in crowded or old cultures. The GP-form, a modified pyriform shape, has been described earlier (see Part III). Various modifications of boomerang or banana forms also have been noted. Gourd-shaped forms with ciliary rows twisted into diagonal orientation are fairly rare. Temporary distortion of shape is very common, owing to the extreme pliability of the pellicle. In the living state in liquid media the anterior tip of the body may be inclined ventrad to a slight degree but I have never observed retention of this in fixed material. A remarkably flattened disk-shape is assumed by the ciliate when cultured on agar media; this has also been observed by Browning (1951) and Browning, Swinford & Varnedoe (1950).

Life cycle. Simple, exhibiting only limited polymorphism (see Part V).

Body size. Range in length, based upon study of both living and fixed specimens, 26–92 μ; mean length times width, $51 \times 31\,\mu$.

Cytoplasmic inclusions. See descriptions by Maupas (1883), Fauré-Fremiet (1906, 1910), Furgason (1940) and Browning (1951). Average size of food vacuoles in bacterized cultures: 3·5–4·5 μ in diameter.

Body ciliation. Cilia are 5–8 μ in length, of considerable thickness, and are inserted into the pellicle closer together at the anterior end although they are not appreciably shorter in length there. No 'caudal bristles' observed. Cilia are placed in the pellicle at intervals along the longitudinal body meridians, none appearing anterior to the first intermeridional connective or posterior to locations of the cytoproct and contractile vacuole pores.

Nuclear components. Macronucleus easily seen in both living and fixed material. It is ovoid to irregularly spherical, averaging about $9{\cdot}5 \times 11\,\mu$ (in haematoxylin preparations), and lies across the central portion of the body, often a little posterior to the midline. Typical macronuclear appearance is revealed in specimens fixed in hot Schaudinn's fluid and stained with iron haematoxylin. The delicate nuclear membrane shows a number of deeply staining granules of slightly varying size adhering to it, giving its inner edge a serrated appearance. The nuclear substance, typically contracted away from the membrane, is densely but quite uniformly granular. Extrusion of chromatin granules during binary fission, typical for all *Tetrahymena* species which I have studied to date, is best revealed in Feulgen preparations; it has been described in detail by Furgason (1940) for *T. pyriformis* T (see also Part IX of this paper). The brief report by Painter (1945) that the macronucleus in strain T–P possesses 'normally coiled and otherwise orthodox chromosomes' warrants further investigation.

Micronucleus is absent in many strains of this species and perhaps in all the axenic strains included in the present study (see Part IX).

Structures revealed by silver impregnation. Descriptions of the following anatomical features are based principally upon statistical study of more than 5000 silver impregnated specimens of the species. It is hoped that the data presented below in some detail may serve a double purpose: not only to indicate the extent of variability in these characteristics within the limits of a single species but also, at the same time, to serve as evidence of the actual

constancy and conservatism of 'silverline' structures and as proof of the dependability and reliability of silver impregnation techniques. Most of the features described in the following pages have been figured in an introductory paper (Corliss, 1952*a*); for orientation, a semi-diagrammatic picture of structures at the apical end of *T. pyriformis* is given in Fig. 5. The contractile vacuole pores and the cytoproct, the principal features of taxonomic importance at the posterior end, may be seen in Figs. 6–11.

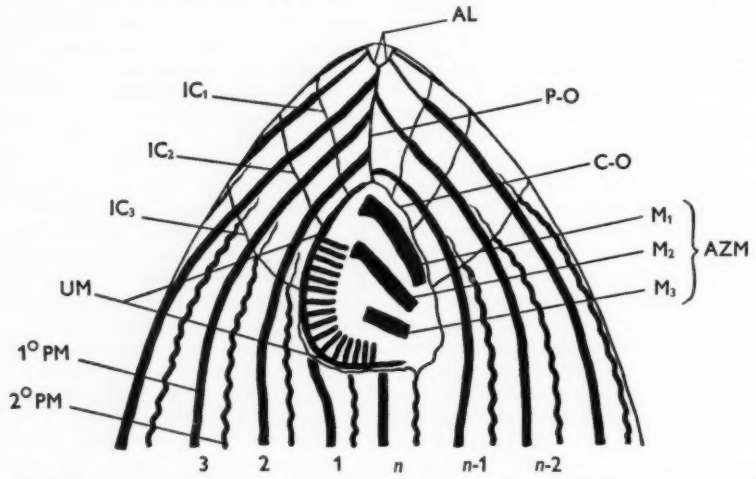


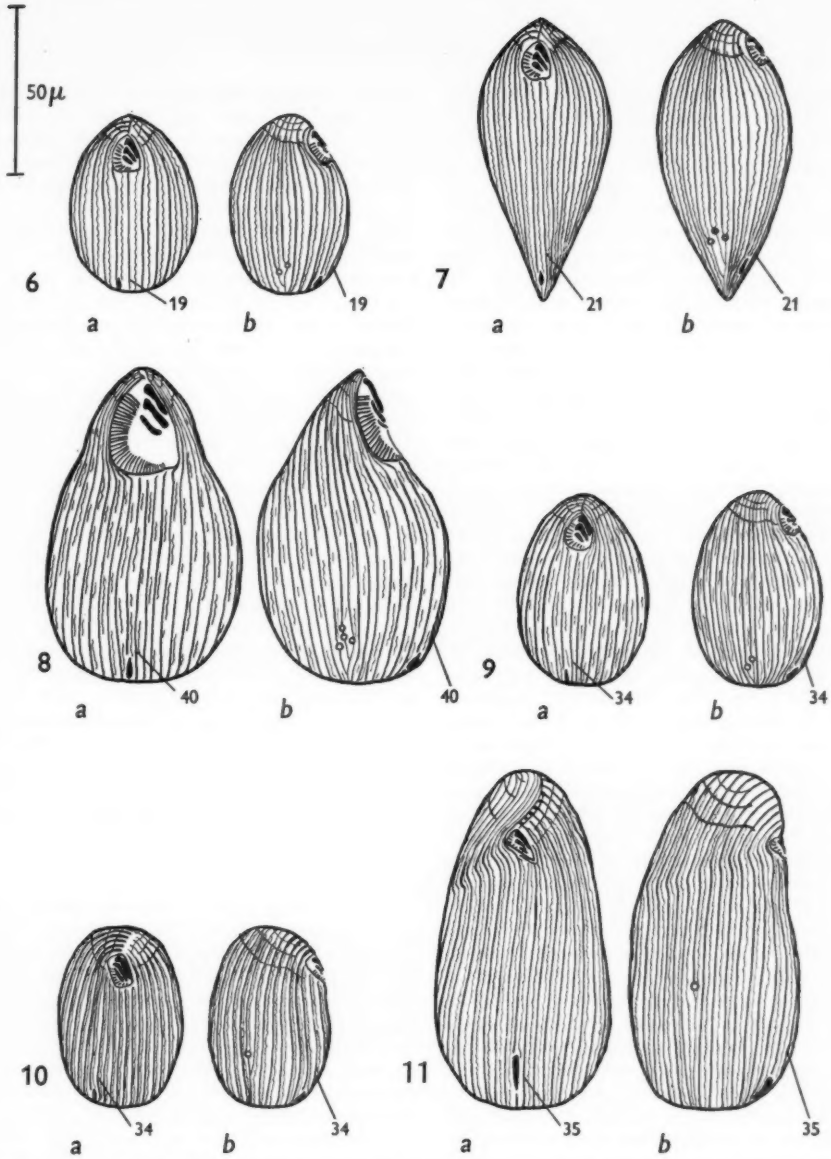
Fig. 5. Semi-diagrammatic representation of buccal and apical structures visible in ventral (and slightly apical) view of specimens of *Tetrahymena pyriformis* (Ehrbg. 1830) Lwoff, 1947, heavily impregnated with silver by the Klein dry method. Minute fibrils in the buccal cavity, intrameridional cross-fibrils and basal granules of cilia have been omitted. See text for detailed description and for explanation of abbreviations.

Buccal overture. Pyriform with right margin slightly more rounded; generally directly in body axis; considered ventral in position on body. Size range, 8·5–12 μ in length; average size, 10·5 × 6 μ. Encircled by delicate fibril, circumoral ring (C-O), difficult to detect in its entirety. Small 'ectoplasmic flange' (Furgason, 1940) along left buccal margin, generally invisible in silver preparations. Distance anterior tip of mouth from apex of body, 4–7 μ, averaging about 5 μ. Number of meridians terminated by posterior or lateral borders of mouth, two or three, typically two. Possesses characteristic tetrahymenal type of ciliary apparatus, described below. (On several occasions specimens have been noted which possessed two mouths; in the majority of these cases the individuals were true homopolar doublets. Spontaneous occurrence of such forms in normal cultures is of interest from the viewpoint of regulation and morphogenesis but further discussion is beyond the subject of this paper.)

Adoral zone of membranelles (AZM). Membranelles three in number (M_1, M_2, M_3) attached to left dorsolateral wall of buccal cavity, M_1 furthest to the left. Composed of cilia considerably longer than body cilia. Bases made up of 2–4 rows of granules and oriented at angles of 20–75° to axis of buccal overture, the average normal disposition being about 30–45° for M_1 and M_2, 50–60° for M_3. Bases of M_1 and M_2 range 5–11 μ in length, M_3 generally very much shorter. Number of delicate fibrils are associated with the membranelles—two make contact with base of M_1, apparently continuations of intermeridional connectives IC_1 and IC_2; another, an extension of IC_3 through C-O ring, branches to make contact with all three bases and with UM base and with posterior portions of C-O ring. Still other shorter fibrils join AZM bases to each other and to UM and to right-hand portions of C-O ring. Pattern of these minute fibrils is actually not constant, rarely are all visible in single specimen, and tracing them out is tedious work.

Undulating membrane complex (UM). Undulating membrane, as seen in living specimens, lies on right-hand border of buccal cavity. In the literature it is generally disposed of by the statement that it is 'composed of a single row of fused cilia'; however, it appears to be a much more complicated structure when considered in its entirety. The following should be considered as a preliminary description since more study, including intensive use of phase microscopy, is required for a more complete understanding of the entire UM complex.

In an investigation of *Tetrahymena patula*,



Figs. 6–11. Representative forms in the genera *Tetrahymena*, *Glaucoma* and *Colpidium*. The semi-diagrammatic figures, all drawn to the same scale, portray characteristics of diagnostic value as revealed in silver impregnated specimens. For each species, *a* shows the ventral aspect of the organism and *b*, the right lateral. Total number of meridians is indicated by numbering the *n*th or last one. To be especially noted are the differences in general body shape, in the buccal structures, and in the number and location of the contractile vacuole pores. The discontinuity of secondary meridians which exists in species *Tetrahymena patula* and *T. faurei* is indicated diagrammatically by fragmentary lines. Figs. 6*a*, *b*. *Tetrahymena pyriformis*. Typical specimens from a bacterized culture, representative of the twenty-one axenic group 'A' strains. Figs. 7*a*, *b*. *T. vorax*. Common tailed microstome form of strain V_2 of group 'B', from a bacterized culture. Figs. 8*a*, *b*. *T. patula*. Average macrostome form of strain L-FF of group 'C', from a bacterized 'zweigliedrige Kultur' containing *Colpidium campylum* as prey. Figs. 9*a*, *b*. *Tetrahymena faurei*. Typical pyriform stage of strain FF-51 of group 'D', from a bacterized culture containing larval chironomid intestine as food. Compare with Pl. 2, figs. 8 and 10, in Kozloff's (1946) paper on a ciliate which I consider to be identical with present strain FF-51. Figs. 10*a*, *b*. *Glaucoma scintillans*. Common appearance shown by members of the bacterized strain designated as M in the present paper. Figs. 11*a*, *b*. *Colpidium striatum*. Typical specimen from bacterized culture of strain NY.

Fauré-Fremiet (1948) briefly suggested that the UM be considered as composed of three parts, here designated as UM_1, UM_2 and UM_3. UM_1 is 'une bande interne' located against the right wall of buccal cavity and perhaps is present only during stomatogenesis. UM_2 is 'une zone intermédiaire à striation radiaire' also located against right wall of mouth pit and extending, presumably, out to external border or rim of buccal opening. In *T. pyriformis* UM_2 generally shows clearly in silver preparations as crescent-shaped area of short, black, parallel rods, 15–25 in number, bordered externally by a heavy 'line' which sometimes appears to be composed of regularly spaced granules. In most preparations these 'ribs' do not exceed 2μ in length; in very favourable material they appear to 'sink in' over an invisible 'ledge' and then extend dorsad and slightly posteriorly into the mouth pit for an additional 4μ. UM_3 is 'une membrane vibratile bordante externe', the part of the UM complex easily seen in the living organism and the entire 'undulation membrane' of the literature. It appears to be composed of a single row or sheet of fused cilia; the individual cilia, about 12μ in length, are rarely revealed by silver impregnation techniques.

Some workers have considered the rib-like structures (UM_2) to be the cilia of the UM proper (UM_3). In brief, support for this notion lies in these facts: number of rods does seem to correspond to number of cilia in the fused membrane; rods appear to have arisen from a row of basal granules; rods lie in a position which could easily be assumed by UM_3 if it were to fall into the buccal cavity upon fixation; cilia do have central cores or axial filaments which could conceivably be impregnated by silver, giving the striated appearance typical of what is here termed UM_2. On the other hand, there is evidence that the rods serve some sort of skeletogenous role and are not parts of the external UM_3 at all: length of rods seems too short, cilia of UM_3 being about 12μ long and UM_2 rods 7μ at most; rigidity of area of rods is such that none ever appears dislocated, not what would be expected of the easily torn UM_3; body and AZM cilia rarely take silver stain and, when they do, they never resemble the UM_2 rods. Resolution of the problem must await further investigation. The one-to-one correspondence in number of rods in UM_2 and of cilia composing UM_3 may indicate a common origin, in the stomatogenous field, from basal granules induced by their surrounding molecular environment to play a dual role in morphogenesis.

Apical loop (AL). Delicate, non-granular fibril encircling a round to ovoid shaped area, not greater than $2–3\mu$ in diameter, at morphological apex of animal. In some specimens, not entirely closed but opened ventrally toward P-O suture region. Sometimes bisected by delicate cross-fibril.

Pre-oral suture (P-O). Considered by Furgason (1940) to be single fibril connecting AL to C-O ring and joined by never more than two meridians. I have found a great deal of variation, however. Sometimes fibril is double in nature, two strands running a parallel course $1–2\mu$ apart; in a few cases, there seems to be no fibril. Average length of P-O suture, $3–5\mu$. A considerably greater number of meridians appear to converge onto suture than reported by Furgason; I have noted four to six with four and five most frequent, in variety of arrangements. Since these patterns are of taxonomic importance, they will be briefly described. When there are four: typically, meridian n-1 progresses anteriorly along left side of cytostome, turns abruptly to right just anterior to mouth, joins P-O suture very near latter's distal termination; n-2 parallels n-1 at level of mouth, continues forward, joins P-O suture near its proximal end less than 1μ from AL; meridians 2 and 3 run anteriorly on right side of mouth, turn gradually to left, meet suture along portion lying between points of contact of left-hand meridians n-1 and n-2. When there are five meridians involved (as shown in Fig. 5): typically, arrangement is as above except that one additional meridian contacts P-O suture; it may be meridian 4, terminating on suture at point very close to AL, or meridian n-3 joining from opposite side. In latter case, much rarer, both n-2 and n-1 turn sharply mediad just anterior to buccal overture. When there are six meridians: either 4 and n-3 join suture in addition to meridians of typical '4-pattern', described above, or the arrangement is as given for '5-pattern' with single extra meridian joining somewhere from either right or left side of body.

First intermeridional connective (IC_1). Most anterior of three delicate transverse fibrils roughly concentric about AL. IC_1 lies only $2–3\mu$ from AL and may be considered approximately at level of anterior margin of M_1. Occasionally it can be seen joining C-O ring and, by very delicate extension, base of M_1.

Second intermeridional connective (IC_2). Generally situated closer to IC_1 than latter is to AL. Located approximately at level of posterior edge of M_1 to which it is occasionally seen to join.

Third intermeridional connective (IC_3). Roughly concentric about IC_1 and IC_2, lying $8–10\mu$ from AL; distance between IC_3 and IC_2 more than double that between IC_2 and IC_1. IC_3 approximately at level of posterior margin of M_2 and anterior margin of M_3. Fibrils may extend from it, at its ventral junction with C-O ring on left side, to bases of M_2 and M_3 although rarely detectable.

Ciliary meridians. Range in number, 17–22, although upper limit noted in only two axenic strains (cf. Table 2), and there rarely; mode, 19–20. Counts made principally on apical polar views in which all bipolar meridians are visible in single

focal plane. Typical number of unipolar or post-oral meridians (POM's) is two with anterior ends terminating directly at posterior margin of mouth or, in case of meridian 1, very short distance up the side of buccal opening. Occasionally three POM's: meridians 1, *n* and *n*-1, last stopping half-way up left side of mouth; or 1, *n*, and a truly 'extra' meridian wedged in between, clearly present at posterior end of body but progressively 'squeezed out' before reaching level of mouth.

There are two main portions to each meridian in this species, a primary portion (1°PM) and a secondary portion (2°PM); the former is much more prominent and is heavily granular as it bears, or appears to bear, the basal granules of the body cilia; the latter is a finer fibril. Posteriorly, 2°PM's unite with their respective 1°PM's just before irregular convergence of meridians in polar region. Anteriorly, 1°PM's retain heavy, granular appearance through IC_3 and IC_2 but those which will continue poleward to AL become reduced to a fine fibril near contact with IC_1; anterior portions of meridians reaching P-O suture, only slightly reduced, are noticeably thicker in those coming from right side of body. The 2°PM's frequently do not extend anterior to level of IC_3 but turn to right near this level, although apparently not joining their 1°PM's; delicate extensions of some 2°PM's occasionally observed as far apically as IC_1. In addition to typically dual nature of meridians, two other extremely delicate longitudinal lines have been seen in favourable specimens. One, here designated 3°PM, appears between 1°PM and 2°PM of same meridian, somewhat closer to latter. It does not seem to be part of or connected to intrameridional cross-fibrils characteristically extending out to left from 1°PM's. The second lies between 2°PM of one meridian and 1°PM of adjacent meridian, much closer to latter; it quite likely represents the 'cinétodesme' of Chatton & Lwoff, a structure rarely impregnated with silver in holotrichous ciliates of the kind under present consideration.

Structures in stomatogenesis. New buccal structures which are formed for opisthe prior to pinching in of body in binary fission originate in a proliferation of granules of meridian 1 out to left. Resulting granular field, located subequatorially, eventually encroaches upon meridian *n*. Oblique bands of granules, anlagen of tetrahymenal buccal ciliature, are formed and assume definitive positions as buccal cavity is developed. (For details, cf. Chatton, Lwoff, Lwoff & Monod, 1931, who studied the phenomenon in strain GL, and Furgason, 1940, who studied it in T.) When anterior mouth has undergone dedifferentiation, as sometimes happens preceding fission, second field of granules originates from meridian 1 at level just posterior to location of former mouth, i.e. at head or anterior end of stomatogenous meridian. In such cases, anlagen develop into new buccal organelles for proter in identically same manner and at exactly same time as do those for opisthe.

Basal granules of cilia (BG's). Duality of these organites difficult to perceive in specimens impregnated with silver by Klein dry method as employed by the writer; neatly revealed, however, in favourable material treated by Chatton-Lwoff technique. Following terminology and interpretation of French investigators, BG's may be said to be composed of kinetosome or blepharoplast proper plus satellite body or kinetoplast.

Cytoproct (CYP). Always located in meridian 1 very near posterior end of organism; when detectable, appears as delicate line, actually a slit, lying more or less between 1°PM and 2°PM. Posterior to it, meridian 1 is represented by single, non-granular, non-cilia-bearing fibril. CYP averages 7 μ in length and generally lies about 6 μ from end of body, end of organism, or posterior pole, being defined as centre-point of irregular posterior convergence of all meridians. When distended, opening of CYP may attain width of 7 μ.

Contractile vacuole pores (CVP's). Typically two, averaging 0·9 μ in diameter, generally located in meridians 5 and 6, at distance of 6–14 μ from posterior pole of body. Exact arrangement of CVP's and variations in a basic pattern have not been described in the literature for this species; such information is offered here because of the increasingly important role these structures are likely to play in future systematics of various ciliated protozoa.

When two CVP's are present: one is located in or just to left of 1°PM of meridian 5 with 2°PM skirting left side of pore and joining 1°PM 1–2 μ further posteriorly. Single granule-free fibril continues posteriorly, often but not always converging with similar fibril of meridian 6 before reaching region of irregular meridional convergence. Second CVP is located in or just to left of 1°PM of meridian 6 with 2°PM of that meridian contacting 1°PM at level of or just anterior to pore. It is nearly always located at level posterior to that of first pore. Non-granular fibril extending posteriorly may or may not converge with that of meridian 5 and/or of 7 before reaching posterior end of body. No cilia present on portions of meridians 5 and 6 posterior to CVP's. Common variations include cases in which one or both CVP's lie directly in 2°PM of their respective meridians; cases in which convergence of 1°PM's and 2°PM's occur anterior, or posterior, to CVP's in both meridians; cases in which posterior convergence of 1°PM and 2°PM, especially in meridian 6, does not occur for considerable distance posterior to pore. When three CVP's are present, not commonly the

situation in pure culture strains of this species: location is generally in meridians 5, 6 and 7 in various positions with regard to 1° PM's and 2° PM's of respective meridians. Convergence of single, non-granular meridional fibrils in such cases occurs only short distance posterior to pores.

Average diameter given above for CVP's is that of entire structure. Actually, pore-opening is considerably smaller since it is supported by relatively heavy wall. Black ring commonly seen in silver preparations represents outer edge of this wall. In very favourable preparations, central black 'dot' can be detected; this indicates location and size of minute pore-opening itself.

(ii) *Species* 'B' (*Tetrahymena vorax*)

Habitat. Fresh-water ponds.

Food. Probably normally bacteria in nature although organisms are capable of becoming carnivorous, devouring other ciliates including members of their own species; may be cultured axenically in autoclaved, non-particulate media.

Conjugation. Never observed. Cannibalism, a phenomenon possibly related to conjugation according to some protozoologists, does occur.

Cyst formation. Never reported.

Body form. Shape varies with stage in life cycle, i.e. with environmental conditions. Tailed microstome stage: elongate, anterior end bluntly pointed, posteriorly distinct tail process variable in shape and length. When starved, body elongation is accentuated, tails tapering to very sharp tip. Pyriform microstome stage: pyriform to ovoid to cucumber-shaped, indistinguishable from forms of *T. pyriformis* described in preceding section. Macrostome stage: broadly pyriform with rounded, enlarged posterior end due to presence of many large food vacuoles.

Life cycle. Can be markedly polymorphic (see Part V).

Body size. Tailed microstome stage: range in length, 31–115 μ; mean length times width, $82 \times 40\,\mu$. Pyriform microstome stage, combining data on all three axenic strains: range, 28–80 μ; average size, $51 \times 30\,\mu$. Macrostome stage: range, 67–140 μ; average size, $103 \times 69\,\mu$. Kidder *et al.* (1940) reported lengths up to 250 μ for this stage in extinct strain D.

Cytoplasmic inclusions. In general similar to those in species 'A'. In carnivorous forms, sizes of food vacuoles range up to 35–40 μ in diameter; number present, in cannibals, generally not over 5; in non-cannibals, 3–20, average about 10. Kidder *et al.* (1940) recorded maximum of 50 for strain D.

Body ciliation. Cilia are 6–8 μ in length, no apparent differences between those of microstome and macrostome stages. Distance between adjacent cilia increased in macrostome forms. See preceding species for further details on ciliation.

Nuclear components. Macronucleus: in all microstome forms, identical with that described for *T. pyriformis*. In macrostome stage, also similar but of greater size, averaging about $25 \times 22\,\mu$ in specimens raised in 'zweigliedrige Kultur' with a strain of *T. pyriformis*. Micronucleus: absent in all three existing axenic strains. Kidder *et al.* (1940), on other hand, reported its presence in extinct strain D. (See Part IX.)

Buccal overture. Pyriform with right margin slightly more rounded; greatly enlarged and relatively broader in macrostome stage; directly in body axis, ventral. Ectoplasmic flange along left margin. Length range in tailed and tailless microstome forms, 8–13 μ; average size, $10{\cdot}5 \times 5{\cdot}5\,\mu$. Length in macrostome forms, 17–43 μ; average size, $29 \times 23\,\mu$.

Adoral zone of membranelles. As in preceding species. Even in macrostome forms, range in lengths of M_1, M_2 and M_3 is not much greater than in *T. pyriformis* although means are higher. In transformation from microstome to macrostome form, sizes of membranelles do not increase in nearly same proportions as do UM complex and buccal cavity itself. In mature macrostome form, M_1 is often located in usual position of P-O suture, which has disappeared, and its forward end lies nearly inside now opened AL.

Undulating membrane complex. Discussed in detail under species 'A'. A few additional observations may be made from study of structure in macrostome forms. Rod-like skeletogenous striations of UM_2 are increased in number to nearly 100; are usually 7–8 μ in length, occasionally reaching 17–18 μ and extending cone-shaped deep into buccal cavity. Increase in size of UM complex, from microstome to macrostome stage, is enormous and transformation, involving both de- and re-differentiation, occurs very rapidly; this amazing phenomenon is worthy of much more extensive investigation.

Pharyngeal pouch. Present in macrostome form, presumably associated with development of carnivorous habit. Mode of formation has been described briefly by Kidder *et al.* (1940); from their account and from personal observations on living material, it may be concluded that the phenomenon is essentially like that occurring in *T. patula*, as graphically described by Maupas (1888).

Apical loop. Identical with that described for *T. pyriformis*, although proportionately larger and generally open ventrally in macrostome forms.

Pre-oral suture. For microstome forms, again see *T. pyriformis*. In mature macrostome forms, two interpretations possible: that suture is entirely absent, as such, or that it consists of two fibrils very close together at opened end of AL, progressively divergent posteriorly. It is toward this opened

path that AZM is moved during formation of macrostome mouth.

Intermeridional connectives. Essentially as in preceding species; proportionately further apart in macrostome forms.

Ciliary meridians. Microstome forms: range in number, 17–23; average, 19–21. Macrostome forms: range, 19–26; average 21–23. Usual number of POM's in both forms, two. Apparent total of four POM's in many mature macrostome forms results from encroachment by broad pocket-like extension of buccal cavity and opening onto meridians which normally lie, undisturbed, to the left. Often bits of anterior parts of what have seemed to be two 'new' POM's may be found very close together on extreme left-hand edge of buccal cavity.

Complex nature of meridians has been discussed in section on *T. pyriformis*, but several additional longitudinal fibrils have been noted in present species. In macrostome forms, more favourable for detailed study because meridians are larger and also more widely spaced, 1°PM's, with attendant intrameridional cross-fibrils (IF's), and granular but non-cilia-bearing 2°PM's are always clearly present; frequently 3°PM fibril is in evidence; more rarely, the kinetodesma. In addition, two unnamed delicate fibrils have been detected: one runs along tips of IF's, but much thinner than these; second lies between 3°PM and 2°PM. Neither of these fibrils are often continuous or unbroken for more than 8–10 μ in length; the possibility that they are consistently formed artifacts cannot be positively denied.

Structures in stomatogenesis. Comparable with those described for *T. pyriformis* whether the phenomenon is occurring in individuals of either microstome or macrostome stages.

Cytoproct. Microstome forms: as described for species 'A', although distance from posterior pole of body is greater in tailed forms. Macrostome forms: only essential difference is in size, lengths of 12 μ having been frequently noted.

Contractile vacuole pores. General location, disposition, and size similar to those of *T. pyriformis*. However, except in forms in irreversible pyriform microstome stage, in which all CVP features are completely identical with those of species 'A', number of pores and which and what number of meridians are involved vary, although within limited range. Tailed microstome forms possess 2–6 CVP's, usually 2–3, located in meridians 5–9; they lie 7–30 μ from posterior end of body, average being 18 μ. Number can be correlated with body size; distance from posterior pole, with length of tail process. Over twenty different arrangements of CVP's in their respective meridians have been observed. Variations are usually of minor nature involving such matters as number of pores in single meridian (never exceeds two), exact disposition of 1°PM's and 2°PM's with respect to pores, juxtaposition of pores, and types of convergence of non-cilia-bearing fibrils posterior to level of CVP's. Recognition of range of variation possible within fairly stable pattern is important in evaluation of pores as criteria of taxonomic significance.

Macrostome forms, both cannibalistic and non-cannibalistic carnivores, possess 2–6 CVP's, usually 5, located in meridians 5–10, usually 6–9; they lie, on average, 16 μ from posterior end of body. Diameter range, 0·9–1·8 μ; average 1·5 μ. Variations in arrangement similar to those noted above for tailed microstome forms.

(iii) *Species* 'C' (*Tetrahymena patula*)

Habitat. Fresh water. Easily found in vicinity of watercress, according to Maupas (1888, 1889).

Food. Reported as normally carnivorous. Except for mouthless stage, it is likely that microstome forms are bacteria-feeders in nature. Both large and small forms thrive axenically in autoclaved particulate media, and macrostome forms, at least, are culturable in same non-particulate media routinely employed for stock cultures of *T. pyriformis* and *T. vorax*.

Conjugation. Reported for microstome forms by Maupas (1888, 1889) who pointed out, however, that conjugants must come from diverse clones. As far as I have been able to determine, the phenomenon has never been described elsewhere in the literature. Because of lack of success in locating additional strains of this species, I have been unable to investigate the possibilities myself.

Cyst formation. Protective or resting cysts never reported but reproductive cysts have been described very briefly (Fauré-Fremiet, 1949). Neither their exact structure nor the precise cause of their formation is yet known. I have observed their occurrence under conditions of starvation, i.e. absence of other ciliates as prey, in boiled water (cf. Fauré-Fremiet, 1949) and also in bacterized cultures supplied with larval chironomid intestine. In the latter case it is not entirely clear whether the ciliate ingests some of the intestine or just the rich bacterial flora soon available there. Reproductive cysts may be seen inside as well as outside the intestine, reminiscent of the situation occurring with certain species of histophagic ophryoglenids studied by Mugard (1948). Although the secreted cystic membrane is fragile and practically invisible, probably mucoid in nature, it is strong enough to confine actively squirming and tightly circling tomites for several hours.

Body form. Macrostome form: broadly and irregularly pyriform, bag-shaped; anterior end bluntly pointed, and anterior third of body scoop-like

in side view appearance owing to convexity of dorsal surface and flatness of ventral surface in buccal region; posterior end plump, hemispherical. Microstome forms: typically pyriform, occasionally cucumber-shaped to cylindrical. Upon commencement of transformation to macrostome form, shape is rounded and full; then anterior half, bearing increasingly larger-sized buccal overture appears separated from smaller, rounded posterior end by slight waist-like constriction. Posterior end next fills out and waist appears to be located further anteriorly as result; nearly all evidence of this partial constriction obliterated as mature macrostome form attained.

Life cycle. Markedly polymorphic (see Part V).

Body size. Macrostome form: range in length, 75–110 μ; average size, 95 × 54 μ. Microstome form: range, 32–55 μ; average, 45 × 28 μ. Furgason (1940) reported length of 80–160 μ for the species; Fauré-Fremiet (1948), 125–150 μ.

Cytoplasmic inclusions. Not unlike those of preceding species. See Maupas (1888). Park (1929) studied osmiophilic bodies associated with the macronucleus; Villeneuve-Brachon (1940) included a brief report on certain 'Golgi' bodies in this species.

Body ciliation. See species 'B'. Note, however, that longitudinal rows of cilia are necessarily set closer together in present species due to nearly double the number being present as compared with *T. vorax*.

Nuclear components. Macronucleus: irregularly ovoid; comparable in size, position, and other characteristics with that found in macrostome and microstome forms of preceding species. Maximum size in macrostome specimens raised in 'zweigliedrige Kultur' with *Colpidium campylum*, 40 × 27 μ. Micronucleus: typically one, embedded in macronucleus; 1·5–3·5 μ in diameter, depending upon stage in life cycle. Experimentally, individuals may be obtained possessing 0–6 micronuclei (Fauré-Fremiet, 1948). Maupas (1888, 1889) mentioned difficulty in detecting micronucleus of microstome forms; it is always present there, however, in Feulgen preparations which I have studied.

Buccal overture. Broadly pyriform with right margin slightly more rounded in macrostome form; typically pyriform in microstome forms (cf. preceding species) although reduced to mere slit in mouthless stage. Ventral. Directly in body axis. Ectoplasmic flange along left margin. Mean sizes: in macrostome form, 29 × 21 μ; in microstome form, 10 × 5 μ.

Adoral zone of membranelles. Macrostome form: comparable with conditions in similar stage of species 'B' although bases of membranelles may reach greater length. Microstome form: exactly comparable with corresponding stages of species 'A' and 'B'.

Undulating membrane complex. As in preceding species. Concept of tripartite structure, discussed under species 'A', is based on study by Fauré-Fremiet (1948) of conditions in present species.

Pharyngeal pouch. Present in macrostome forms except during binary fission. Its formation has been excellently described, in detail, by Maupas (1888).

Apical loop. Generally open ventrally in macrostome forms, as in species 'B'. Typical appearance in microstome forms.

Pre-oral suture. As in case of *T. vorax*, it may be considered absent or of double nature in macrostome forms; as typically described for corresponding stages of preceding species in microstome forms.

Intermeridional connectives. In all characteristics, as in species 'A' and 'B'.

Ciliary meridians. Macrostome forms: range in number, 36–42; average, 40. Microstome forms: range, 34–40; average, 36–38. Typical number POM's, 4; 3 and 5, rare exceptions. Position of POM's in macrostome form differs from that of corresponding form in species 'B' in that there is no evidence that two most left-hand meridians continue poleward; these do, however, show slight curvature to left at tips, just before contacting posterior border of buccal overture. General nature of meridians in present species not as complex as in *T. vorax*; 2°PM's often appear discontinuous and 3°PM's have not been observed.

Structures in stomatogenesis. Behaviour identical with that noted for species 'A' and 'B'.

Cytoproct. Typical location, size and distance from posterior pole of body as in corresponding forms of preceding species.

Contractile vacuole pores. General location, disposition and size similar to those of *T. pyriformis* and *T. vorax*. Macrostome forms: 2–6 CVP's, typically 4–5; located in meridians 10–15, typically 10–12. Average diameter, 1·5 μ. Distance from posterior end of body, usually 9–11 μ. Microstome forms: 2–4 CVP's, typically 2–3; located in meridians 9–12, typically 9–11. Average diameter, 0·9 μ. Distance from end of body, usually 5–8 μ.

Typical pattern of arrangement of CVP's in macrostome form: typically, one in meridian 10; two in 11, one directly above other, both slightly anterior to one in 10; one in 12, at level posterior to other three. Variation in pattern reminiscent of those noted for *T. vorax*. In microstome forms: as described, in general, for corresponding stages in preceding species; when three CVP's present, two most frequently located in second of two meridians involved, although occasionally one pore in each of three adjacent meridians.

From the viewpoint of how variations in such relatively conservative structures arise in forms which by pinching in two supposedly produce two identical daughter organisms, the following observa-

tion, first noted in present species, is worthy of mention. Disposition, and even number, of CVP's was found to be not necessarily identical in both 'halves' of individual undergoing fission. In study of macrostome forms fixed during actively dividing stage of life cycle, particular attention was given to observation of pattern of pores; in low but definite percentage of cases CVP's of proter, which must be produced as new structures, appeared in different meridians from those bearing CVP's of opisthe, occasionally number of pores also differing.

(iv) *Species* 'D' (*Tetrahymena faurei*)

It has been indicated (cf. Part VII) that the pure culture strain FF-51 was probably identical with the ciliate described by Kozloff (1946) as '*Tetrahymena limacis* (Warren, 1932) comb. nov.' but that both FF-51 and Kozloff's strain differ morphologically from Warren's ciliate principally in the number of ciliary meridians and in pellicular features dependent upon meridian number. In the following description reference will be made to Kozloff's organism as 'strain K' although this strain was never established in axenic culture. The data on FF-51 are based principally upon study of fixed preparations since all cultures of the strain were lost before the writer had an opportunity to observe the organism in life.

Habitat. Strain FF-51: found as facultative parasite in cerebrospinal fluid and tail musculature of embryonic trout. Strain K: discovered infecting liver and other visceral organs of slug *Deroceras agreste*.

Food. FF-51: probably bacteria in free-living stages in nature; capable of saprozoic existence both as facultative endoparasite and as test-tube organism cultured axenically on autoclaved, non-particulate media. K: substances available in host's tissue, in parasitic phase; in laboratory cultured free-living phase, on bacteria, starch granules and particulate matter of slug liver infusions.

Conjugation and cyst formation. Not reported for forms here included in this species.

Body form. FF-51: parasitic phase, ovoid; in laboratory cultures, typically pyriform or distortion of that shape in which anterior part of body is attenuated, causing buccal opening to appear in a position relatively further posterior. K: parasitic phase, typically cucumber-shaped, anterior end apiculate; free-living phase, generally pyriform, anterior end somewhat pointed but not apiculate.

Life cycle. In laboratory cultures, as described for species 'A': simple, exhibiting only limited polymorphism. Changes in shape, termed adaptive polymorphism in present paper, shown in parasitic environments (see Part V).

Body size. FF-51: form with elongate anterior end, range in length, 34–54 μ; average size $44 \times 27\,\mu$ pyriform form, range, 40–68 μ; average, $57 \times 37\,\mu$. Average of both forms, $50 \times 31\,\mu$. K: apiculate form, range, 33–68 μ; average size, $55 \times 27\,\mu$; pyriform, range, 28–68 μ; average, $44 \times 27\,\mu$. Average of both forms, about $50 \times 27\,\mu$.

Buccal overture. Pyriform with right margin slightly more rounded; directly in body axis; ventral. Usual size, $10{\cdot}5 \times 5\,\mu$. Tetrahymenal buccal ciliature, exactly as in three preceding species. In study of living material Kozloff could not detect presence of ectoplasmic flange in strain K; no information available regarding its presence or absence in strain FF-51.

Nuclear components. Macronucleus: composition, shape, location comparable with conditions found among strains of species 'A'. FF-51: average size, $8 \times 7\,\mu$ (Feulgen preparations). K: reported range, $8 \times 6\,\mu$ to $14 \times 11\,\mu$ (Haematoxylin preparations). Micronucleus: single, commonly embedded in macronucleus. FF-51: average diameter, about $2{\cdot}5\,\mu$. K: size range, $1{\cdot}8$–$2{\cdot}4\,\mu$.

'*Silverline*' *structures.* AZM and UM complex and also general arrangements, numbers, sizes and locations of various pellicular structures are comparable with those described in preceding pages for species 'A', 'B' and 'C'. In fact, even in data on distances between minute fibrils (e.g. between IC_1 and AL, IC_1 and IC_2, and the like), the pyriform stage of the present species is morphologically indistinguishable from *Tetrahymena pyriformis* except for the former's possession of a greater number of ciliary meridians and for resulting differences in characteristics, such as location of CVP's and number of meridians converging onto P-O suture, which are dependent on total meridional number. The great taxonomic importance of numbers of ciliary rows is thus well illustrated in the case of the present ciliate.

Range in number of meridians, 32–37; most commonly, 34–36. Number of POM's, 2–3; meridian 1 may terminate short distance up right buccal border. Number of meridians joining P-O suture, 12–15; pattern basically as in species 'A' in that meridian n-1 turns abruptly to right just anterior to cytostome while n-2 continues towards AL and several meridians from right side of body contact suture between levels of n-1 and n-2. The 1°PM's, embellished with usual IF's or 'stiriate serrations' of Kozloff, are typical; 2°PM's, as in case of species 'C', appear incomplete and discontinuous in strain FF-51. Typical location of CYP, in terminal portion of meridian 1, near posterior end of body. Usually 2–3, less often 4, CVP's; in FF-51, located in meridians 8–11; in K, 6–8. Average diameter of CVP's, $0{\cdot}8\,\mu$; distance from posterior pole of body, 5–8 μ.

IX. THE MICRONUCLEAR PROBLEM

The micronuclear situation in certain species of the genus *Tetrahymena*, in particular *T. pyriformis* and *T. vorax*, presents an engima difficult to resolve. Whether the micronucleus, classically 'indispensable' in all true ciliates which, indeed, are set apart taxonomically by the duality of their nuclear apparatus, is always present but sometimes invisible, or is as often absent as present, or is present in early years of laboratory raised clones, disappearing with time, poses a problem regarding answers to which our present information is fragmentary.

Brief historical consideration of the occurrence and significance of amicronucleate ciliates will be advisable before focusing attention on the situation existing in pure culture members and related bacterized strains of the C-G-L-T group. Since the turn of the century there have been some fifteen reports of discovery of naturally occurring amicronucleate races of species in nine or ten genera; most of these cases are cited by Woodruff (1921) and Beers (1946). It is worth noting, however, that these races have been the exception, not the rule; i.e. the strains were members of species which normally have been described in the literature as possessing easily observable micronuclei. The significance of such cases, as several investigators who have worked on either naturally occurring races or experimentally produced amicronucleate strains have pointed out, lies in their revelation of the relatively much greater importance of the macronucleus in the lives of the animals involved. Not only do the amicronucleate individuals, in nearly all cases, survive but they also grow and multiply with typical vigour and are able to carry out all the so-called vegetative aspects of life, including such diverse functions as locomotion, food capture, growth, en-and ex-cystment, reproduction by binary fission, maintenance of cell proportions and form, and possibly regeneration. Naturally the organisms are unable to carry to completion such processes as autogamy, endomixis and conjugation. (For fuller accounts, cf. especially the papers by Balamuth, 1940; Beers, 1946; Horváth, 1947; Grell, 1950.)

With regard to pure culture members of the C-G-L-T group, Furgason (1940) was the first investigator to call attention to the possibility of an entire species being amicronucleate, as opposed to the occurrence of an occasional or aberrant strain showing that condition. Working with *T. geleii* (= *T. pyriformis*), strains T, E, H, and one now extinct, he demonstrated clearly the complete absence of any micronucleus in these organisms. He pointed out the danger of confusing 'micronucleus-like bodies' with true micronuclei, suggesting that chromatin extrusion from the macronucleus was the probable source of the former. Some time ago I examined all twenty-one group 'A' strains with a standard iron haematoxylin technique and I could not determine with certainty the absence of micronuclei in any of the ciliates because of the relatively non-specific nature of the haematoxylin stain. Very recently I have restudied fourteen of the axenic strains using the Feulgen nucleal reaction and employing both 18–20 hr. cultures, rich in dividing forms, and 5–7-day old cultures, in which the organisms are in the maximal stationary phase of growth. As a special control, I used cultures of the same ages of a very closely related species* of practically the same body and macronuclear size; furthermore, this second species, to be described in a later paper, also exhibits typical macronuclear chromatin extrusion.

The fourteen strains (GP, Ch-S, L-I, L-II, T, T-P, Gf-J, GC, W, GL, H, E, S and Gl-R) appear to be 100 % amicronucleate. There are two types of 'pseudo-micronuclei' the true nature of which can always be determined under oil immersion: extruded macronuclear material and condensation of a small sphere of more intensely staining chromatin at the centre of the macronucleus; the latter, nicely resembling an embedded micronucleus under low magnifications, is formed early in the process of binary fission of the organism and its appearance is typical for a number of species of holotrichous ciliates. In some specimens the rounded-up mass of Feulgen-positive substance extruded during the seemingly amitotic division of the macronucleus can, in size and position, resemble a micronucleus to a striking degree; not only are such specimens rare, however, but also the 'life cycles' of such chromatin masses can be traced by examination of thousands of specimens. These 'micronucleus-like bodies' are not constant in position or in number, often being completely absent; they are generally irregular in shape and often larger than micronuclei; they never show mitotic figures; and they frequently fade in staining intensity with time. The micronuclei in my control animals, members of a closely related species, on the other hand, are typically embedded in the macronucleus (although easily visible with Feulgen technique) except during and immediately following binary fission; they are very rarely absent, being typically single in number when the cultures are not at an actively dividing stage; they are, in the resting state, of a constant, round-to-oval shape with little variation in size; they exhibit mitotic figures during binary fission; and they always show a strong cherry-red colour of an even intensity.

Whether the remaining seven axenic strains of *T. pyriformis*, which I have not yet had an opportunity to study with the Feulgen technique, are amicronucleate cannot be stated until they have

* *Tetrahymena parasitica* (Penard, 1922) Corliss, 1952.

been carefully examined. It is not unreasonable to suspect that many if not all of them are also without micronuclei.

In addition to the investigation of axenic strains, I have also examined critically the nuclear situation in seven strains of this species which have been isolated from fresh-water sources in the region of Paris and have been established in bacterized cultures. The ciliates have been found to be totally amicronucleate. Very recently I have isolated eleven strains found as parasites in the body cavity of the larvae of *Chironomus plumosus* (see Part VI); once again, Feulgen preparations revealed the complete absence of a micronucleus. The '*Glaucoma piriformis*' of Hoare and Watson (see p. 65, col. 1, n. *), obtained in London, was also found to be amicronucleate. Considering both axenic and bacterized strains, ranging in age as laboratory cultures from 30 years to 2 or 3 days, at time of staining, and originally isolated from nature in three different countries and in a variety of geographical areas (cf. Corliss, 1952*a*), one might be tempted to conclude that the entire species is truly amicronucleate. If the loss occurred at some unknown time in the past history of the ciliate by an unequal distribution of the micronuclei during binary fission, or in some other way, then one would probably be justified in assuming that such an unbalanced segregation of the nuclei is of common occurrence wherever the species has existed, and, of greater significance, that the amicronucleate lines, once established, apparently have a higher survival value under the processes of natural selection.

On the other hand, this species also seems to have been frequently found with its micronucleus present. In some of the older reports in the literature it is possible, of course, that extruded chromatin bodies of macronuclear origin may have been confused with true micronuclei, especially in times when less precise nuclear stains were available. Maupas (1883), however, in his description of the macronucleus in his *Glaucoma pyriformis*, stated definitely, 'Il est toujours accompagné d'un petit nucléole sphérique, sans structure apparente'. Fauré-Fremiet (1906, 1910) also recorded the presence of a micronucleus in this species, observing it undergoing mitosis in the first study. Recently Mugard (1948), using the Feulgen technique, reported one for her bacterized strain of the species, noting that it was never over 1·5 μ in diameter. In Feulgen preparations of a strain which Professor Fauré-Fremiet isolated in 1948 (now extinct) I have found a micronucleus present, almost without exception, in every one of hundreds of specimens examined; average size, slightly over 1·5 μ. In both of the two last-mentioned cases, study of silver impregnation preparations put at my disposal has revealed that the ciliates, both originally collected in the Paris area, were truly strains of *Tetrahymena pyriformis*.

I have very recently studied an organism apparently identical with the *Colpidium glaucomaeforme* of von Gelei (1935) and have also isolated several strains of ciliates which seem, morphologically, to bridge completely the gap between the *Tetrahymena geleii* of Furgason (1940) and von Gelei's animal. All these ciliates are never without a micronucleus and it is doubtful whether this characteristic alone should prohibit their inclusion in the species *T. pyriformis* (cf. section on taxonomy of group 'A', p. 66; a resolution of this particular taxonomic problem is suggested briefly in another paper (Corliss, 1952*b*) and is beyond the scope of the present investigation.

Of interest, but of a significance not understood, is the case of the pure line and axenic strain Ch-S, isolated by Chatton in 1925. About 10 years after its isolation it was figured with a definite micronucleus by Chatton & Tellier (1934); in 1951, however, I found it to be entirely amicronucleate. (Is it possible that rigid culture of a clone over many years in a standardized medium can result in the ultimate loss of an organelle apparently no longer of survival value to the organism? Yet such a notion is seriously undermined by discovery of amicronucleate members of *T. pyriformis* immediately upon isolation from natural habitats or upon release from the body cavity of chironomid larvae, as mentioned above.)

The entire micronuclear problem is further complicated by the case of members of the species *T. vorax*. In 1940 the now extinct pure culture strain D was described from haematoxylin and Feulgen preparations as having 'a single micronucleus, located near the macronucleus' (Kidder *et al.* 1940). There has been no published information on the earlier nuclear situation of extant strains V_1, PP and V_2, isolated from nature in 1940, 1942 and 1947, respectively; in late 1951, however, I found all three to be absolutely amicronucleate. It is interesting to note that whereas PP and V_1 are now morphologically indistinguishable from strains of *T. pyriformis* in all other features as well as in nuclear components, strain V_2, although similarly amicronucleate, is still capable of undergoing the reversible transformation microstome⇌macrostome form. There is not the slightest evidence of a micronucleus in dividing or non-dividing specimens from either stage in the life cycle of V_2. That the micronucleus has been lost in strains of *T. pyriformis* and *T. vorax* cultured under similar conditions may be considered as impressive evidence of the close relationship between the two species (also see Part VII, under species 'B'). *T. patula* on the other hand, established in particulate axenic culture in 1942, still possesses a typical micronucleus, evident

in forms at all stages in the life cycle. Strain FF-51 of *T. faurei* also possessed one at the time of its discovery; subsequent loss of the culture has prevented further investigation of the nuclear situation in this strain.

X. CONSIDERATION OF INVOLVED GENERA AND OF HIGHER CATEGORIES

Although the twenty-six axenic strains of C-G-L-T ciliates included in the present investigation are believed by the writer to be members of congeneric species, the fact that they have been allocated to several different genera in the past must be considered. Furgason (1940), based upon his thorough reinvestigation of the type species of the genera *Colpidium* of Stein (1860) and *Glaucoma* of Ehrenberg (1830) and upon his careful studies of *Tetrahymena pyriformis* and *T. patula*,* presented a table of characteristics from which one may recognize generic limits for these three genera. Some time ago I isolated representative species of the first two genera in order to study them by the same methods used in my work on the various *Tetrahymena* strains; recently I have had an opportunity to make a thorough investigation of a number of additional species in each genus. Figs. 10*a*, *b* and 11*a*, *b*, show the principal differentiating characters; these drawings, based upon my earlier work, were prepared from silver impregnated preparations of bacterized cultures† of *Glaucoma scintillans* and *Colpidium striatum*, designated as strains M and NY, respectively. My study of these ciliates revealed that they fell within the generic limits indicated by Furgason's work and that they differed as much from each other as from members of the genus *Tetrahymena*. To be noted in particular in the figures of representative ciliates of all three genera (Figs. 6–11), are the shape of the buccal overture and its orientation in the body axis, the number of meridians terminating at the borders of the mouth, the shape of the anterior end of the body and the pattern of convergence of bipolar meridians there, and the number and location of the contractile vacuole pores. Among other existing differences, not portrayed, may be mentioned the dissimilarity in size, appearance and body location of the nuclear components. Further consideration of *Glaucoma* and *Colpidium* species is beyond the scope of the present paper.

Other genera which occasionally have been involved in the history of extinct or existing pure culture strains or of closely related members of congeneric species, since Maupas's (1883) careful listing of synonyms of his *Glaucoma pyriformis*, include: *Trichoda* and *Leucophra* of O. F. Müller (1773 and 1780), *Leucophrys* of Ehrenberg (1830), *Saprophilus* of Stokes (1887), *Leucophrydium* of Roux (1899), *Paraglaucoma* of Kahl (1926), and *Paraglaucoma* of Warren (1932). Three additional generic names which further study may show are closely involved are *Lambornella* of Keilin (1921) and *Leptoglena* and *Turchiniella* of Grassé & de Boissezon (1929). As far as the present study is concerned, all these 'genera' have been disposed of sufficiently in Part VII (in particular, cf. p. 66, col. 1, n. †).

Detailed consideration of categories above the generic level will be reserved for later papers. It may be pointed out that Furgason's (1940) classical interpretation of the significance of the pattern of buccal ciliature in certain hymenostome species otherwise so relatively undifferentiated in most other characteristics, and its wide and extended application in the recent thorough investigations by Fauré-Fremiet and his students, may serve as the basis for a rather complete revision of the systematics of all the ciliates. It is already obvious that the genera *Frontonia* and *Tetrahymena* can no longer be contained in the same family, the heterogeneous Frontoniidae Kahl, 1926. Since the tetrahymenal type of buccal ciliature appears to be a basic and perhaps primitive arrangement of these organelles, the establishment of a new family Tetrahymenidae to include at least all the hymenostome ciliates of the C-G-L-T type recently has been proposed by the author (Corliss, 1952*c*). In effect, this name replaces Leucophryidae Mugard, 1948, a family erected without detailed discussion to contain three histophagic species. Mugard obviously had the '*Leucophrys patula*' of Ehrenberg in mind when she proposed her family name, although she did not state so. The name, however, is preoccupied by Dujardin's (1841) 'famille Leucophryens' and is also unavailable if the suggestion that the generic name *Tetrahymena* should replace *Leucophrys* (= *Leucophra*) is accepted (see Part VII, sect. (i)).

XI. SUMMARY

In the last 30 years, at various laboratories in the United States, France and England, many strains

* Furgason, as have other workers in the past, erroneously considered *T. patula* to be the type species of the genus *Leucophrys* (= *Leucophra*); nevertheless, he recognized the close relationship between his *L. patula* and his *T. geleii*. When the full life cycles are considered and the more recently discovered 'intermediate' forms such as *T. vorax* are studied, most of the differences listed by Furgason disappear; those that remain are, in my opinion, of value only below the generic level. In any case, by the rules of nomenclature, *T. patula* can never be type or even a member of the genus '*Leucophrys*' (see Part VII).

† *G. scintillans* was found in a small aquarium belonging to Mr Joseph H. Miller, Department of Biology, University College, New York University; *C. striatum* was isolated from a commercial *Amoeba* culture.

of small holotrichous ciliates have been established in axenic culture for use in a widening variety of investigations of a physiological or biochemical nature. Since few of these organisms have been adequately described or properly identified by the workers employing them, thus making difficult meaningful comparison of the various experimental results reported, the need for morphological and taxonomical study has become increasingly apparent. At least twenty-nine such axenic strains of ciliates, considered members of a '*Colpidium-Glaucoma-Leucophrys-Tetrahymena* group' because it has been principally to these four genera that the protozoa have been assigned in the past, are still in existence and twenty-six of them were collected for use in the present investigation. For accurate identification of such relatively undifferentiated ciliates the use of silver nitrate impregnation techniques, the value of which was recognized some years ago by Klein (1926) and stressed by Furgason (1940), is re-emphasized as being nearly indispensable. Details of buccal organelles and differentiated pellicular structures in other parts of the body are revealed precisely and with constancy.

The majority of the axenic strains under present consideration, allocated to some twelve species in a number of genera in past years, have been best known more recently by the names of *Glaucoma pyriformis* (or *G. piriformis*), based principally on the classical work by Maupas (1883), and *Tetrahymena geleii*, originating in the careful investigation by Furgason (1940). Very recently, the name *Leucophrys piriformis* has been suggested (cf. Lwoff, 1951).

After showing by comparison of data on twenty-six strains that the ciliates, first, may be divided into four groups and secondly, may be regarded as congeneric forms (cf. Parts III and IV), I have concluded that nomenclaturally the only valid and available*generic name is *Tetrahymena* Furgason, 1940, and that the groups of axenic strains should be allocated to the following specific categories (cf. Part VII):

T. pyriformis (Ehrenberg, 1830) Lwoff, 1947 [twenty-one strains, by strain designation and in order of mean body lengths: GP, Ch-S, L-I, LR, EZ, HS, L-II, N, GHH, T, L-III, Gf-J, GC, Y, W, GL, T-P, H, E, S and G1-R].

T. vorax (Kidder, Lilly & Claff, 1940) Kidder, 1941 [three strains: PP, V_1 and V_2].

T. patula (O. F. Müller, 1786) Corliss, 1951 [single strain: L-FF].

T. faurei Corliss, 1952 [single strain: FF-51].

The four species may be differentiated largely on the basis of the total number of ciliary meridians, with attendant variation in involved structures, and upon characteristics associated with the kinds of polymorphism exhibited during their life cycles (cf. Part V). *T. vorax* presents an especially perplexing problem. In its complete life cycle, now demonstrable in only one of the three strains, one stage is strikingly similar to a corresponding stage of *T. patula*; yet in the majority of its morphological features it is entirely indistinguishable from *T. pyriformis*, even in its lack of a micronucleus. The possibility that strains of *T. pyriformis*, 'phylogenetically' speaking, once possessed the ability to undergo the reversible transformation, microstome ⇌ macrostome form, still shown in *T. vorax* when freshly isolated from nature, and that therefore both groups are in reality members of but a single species is discussed (Part VII).

In a later section (Part VIII) full descriptions of the species are offered with particular attention to details of pellicular differentiation as revealed by silver impregnation techniques. Differences in these characteristics, often very slight, serve as evidence of the amount of variation within a single species and between congeneric species and, at the same time, as demonstration of the relative constancy and conservatism of the features themselves, features which thus may be regarded as of value in systematics. Details of the buccal and apical structures present in *T. pyriformis* are shown semi-diagrammatically in Fig. 5.

The life cycles of members of the different species present an interesting series of increasing complexity. *T. pyriformis* exhibits only limited polymorphism, dependent upon amount of food available; *T. faurei* is similar to *T. pyriformis* under laboratory cultural conditions but presents a different body form (described for Kozloff's strain) in its parasitic phase; *T. vorax* is able to undergo a major transformation of form, particularly in the buccal region of the body, in adaptation to the carnivorous, including cannibalistic, habit; *T. patula* is able to undergo a strikingly similar transformation but has a life cycle complicated by the introduction of a temporary reproductive encystment stage. (Cf. Part V and, for the last two species, Figs. 3 and 4.)

The easy adaptability of at least two of the species to facultative parasitism has been shown by observations made on naturally occurring and experimentally induced cases. Some twenty reports in the literature on holotrichous ciliates found as accidental or facultative parasites in a variety of metazoan hosts generally have involved organisms that were quite likely closely related members in the C-G-L-T group, possibly all species of the same genus, *Tetrahymena*, to which the twenty-six axenic strains under present consideration have been allocated. (Cf. Part VI.)

In study of the nuclear complex in the ciliates which have been raised in pure culture, a special

* Pending approval by the International Commission on Zoological Nomenclature of suppression of certain earlier synonyms (see p. 66, col. 1, n. †).

'micronuclear problem' arose. The strains of *T. vorax* and all those of *T. pyriformis* examined to date have been found to be amicronucleate. Bacterized strains of the second species, isolated very recently from fresh-water habitats in the Paris area and from the body cavity of chironomid larvae also were found to be without any sign of a micronucleus. The same condition exists in a coprophilic strain from London. On the other hand, *T. pyriformis* has always been described in the older literature as possessing a micronucleus and two extinct bacterized strains from the Paris area had a typical, although relatively small, micronucleus. Furthermore, one axenic strain, Ch-S, now amicronucleate, was believed to have possessed a micronucleus in past years; also an extinct pure culture strain of *T. vorax* was reported as having a typical micronucleus. The wide geographical distribution of the amicronucleate strains, both axenic and bacterized cases, possibly places *T. pyriformis*, at least, in a different category from the occasionally found amicronucleate races of other ciliates. That their long cultivation as pure clones in standardized medium is the cause of the loss of the micronuclear body cannot be considered the entire solution to the problem, since new strains from nature have been found with the same deficiency. Regarding the consistent reports in the older literature of an ever present micronucleus, the matter is complicated because of the typical macronuclear chromatin extrusion now known to be exhibited by this and related species; such extruded bodies sometimes may have been confused with true micronuclei, particularly in times when only less specific stains were available. (Cf. Part IX.)

The involved genera other than *Tetrahymena* are considered briefly, Furgason's (1940) work on type species having already established clear-cut generic differences among *Colpidium*, *Glaucoma* and *Tetrahymena*. The major differences may be noted in Figs. 6–11 in which representative ciliates of these three genera are depicted. Recent investigations and work in progress by Fauré-Fremiet and his students are concerned with a revision of higher categories. A new family, the Tetrahymenidae, in effect replacing the Leucophryidae of Mugard (1948), has been suggested by the author in a separate paper.

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SHELL GLAND AND EGG-SHELL FORMATION IN *FASCIOLA HEPATICA* L.

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(With Plates I and II and 1 Figure in the Text)

INTRODUCTION

Since the middle of the nineteenth century the shell gland, or Mehlis's gland, of the Trematoda has been the subject of numerous investigations, and many opinions have been expressed concerning its function. Little work, however, has been carried out with the aid of modern cytological techniques.

Blumberg (1871) believed that the egg-shell of trematodes is formed from a secretion of the shell gland. Stiles (1894) examined specimens of *Fasciola magna* (*Fascioloides magna*) and claimed that shell-forming material originates from the vitelline cells in the form of yellowish globules and that the shell gland takes no part in the process. Leuckart (1886–1901) claimed to have observed the origin of shell-forming material from globules situated in the yolk cells. According to him the function of the shell gland is to harden the newly formed shell.

A number of workers studied egg-shell formation in *F. hepatica*. Henneguy (1902, 1906) stated that the yolk cells play the chief part in providing material for the shell. Schubmann (1905) observed that, as the liberation of granules takes place in the more distal uterine coil only, the vitelline cells do not take any part in egg-shell formation. The shells, he believed, arise from the secretion of the shell gland. Taylor (1934) noted the breaking down of vitelline cells in the proximal region of the uterus and that the shell substance is deposited by the yolk cells in the neighbourhood of the shell gland. He believed that the production of malformed eggs is a normal process in the early stages of the reproductive activity of *F. hepatica*. Kouri, Basnuevo, Alvaré, Lescano & Simon (1936) recorded that granules are produced by the vitelline cells and that these fuse and form the shell. Kouri & Nauss (1938) stated that 'in the first portion of the uterus, there are accumulations of spermatozoa. In this same first part of the uterus, outside and at a certain distance from Mehlis's gland, is the site of manufacture of the egg shell, chiefly and probably solely by the vitellogenous cells'. According to these authors vitelline cells supply shell material and food substance. The application of yolk cells to the oocyte takes place in the first loop of the uterus and is followed by the formation of the shell.

Stephenson (1947) claimed that no visible secretion is produced by the shell gland and that the shell is formed from polyphenol granules contained in the vitelline cells. The granules are not released until the vitelline cells pass the uterine valve and reach the distal uterine coils. According to his account the vitelline cells are churned about in the uterus together with ova and spermatozoa. Stephenson believed that mechanical force plays a major part in the liberation of the polyphenol granules. He considered that the formation of the shell from isolated vitelline cells, and the arrangement of these cells around the oocyte, is based upon the activity of the spermatozoa and that the function of the shell gland is to produce a fluid by which the activity of the spermatozoa is increased.

Smyth (1951) recently reported that the shell material of trematodes is coloured a brilliant green by methyl and malachite green. He suggested that these stains are absolutely specific for shell material. This author strongly supports the views held by Stephenson regarding egg-shell formation.

Goldschmidt (1909), Johnston (1912), Tyzzer (1918), Manter (1927), Augustine (1929), Cable (1931), Idris Jones (1933), Anderson (1935), Yumoto (1936), Ujiie (1936*a*, *b*), Murikama (1937), Rees (1939), Dawes (1940), Markell (1943), Willey & Koulish (1950) and Willmott (1950) studied egg-shell formation in other trematodes. The majority of these workers claimed that the vitelline cells are concerned with the formation of the shell. There is, however, considerable difference of opinion as to the function of the shell gland.

MATERIAL AND METHOD

Flukes which showed active signs of life were removed from the livers of sheep obtained from the Belfast Slaughter Houses. Specimens were fixed in Bouin's solution and in Regaud's formol dichromate. Sections prepared according to the methods of Cajal, Da Fano and Aoyama for the study of the germ-cells (Yosufzai, 1952*b*, 1953) were also studied. Material fixed by these methods was quickly rinsed in distilled water and transferred to 1·5% silver nitrate for 72 hr. It was then quickly washed in

distilled water and transferred to freshly prepared reducing fluid for 36 hr.

In all cases transverse and longitudinal sections were cut at 5 μ in thickness. Sections of material fixed in Bouin's fluid were stained in Mann's methyl blue and eosin, in Delafield's haematoxylin and eosin combination or in Heidenhain's haematoxylin. Sections of the material treated according to Regaud's formol dichromate method were stained in Heidenhain's haematoxylin. Silver preparations were toned with gold chloride.

OBSERVATIONS

The shell gland of *F. hepatica* is divided into two zones, a peripheral zone (*P.Z.*) composed of large basophilic cells and a medullary zone (*M.Z.*) made up of radiating protoplasmic strands which converge towards the centre of the gland and possess great affinity for eosin (Pl. I, fig. 1; Text-fig. 1). Granular Golgi bodies are present in the peripheral basophilic cells (silver preparations). The latter possess long funnel-like ducts which converge towards the middle of the shell gland (Pl. I, fig. 2). Silver preparations reveal the presence of an extremely hyaline secretion between the protoplasmic strands; this secretion is apparently a product of the gland cells. The secretion is light yellow in colour and exactly resembles the colour of the newly formed egg-shell. The hyaline secretion is present throughout the entire shell gland, thus indicating that this structure secretes a hyaline fluid which is discharged towards the centre of the gland. Eggs with fully formed shells are present in the proximal part of the uterus which Stephenson (1947) calls the central chamber of Mehlis's gland (Text-fig. 1, *P.U.*).

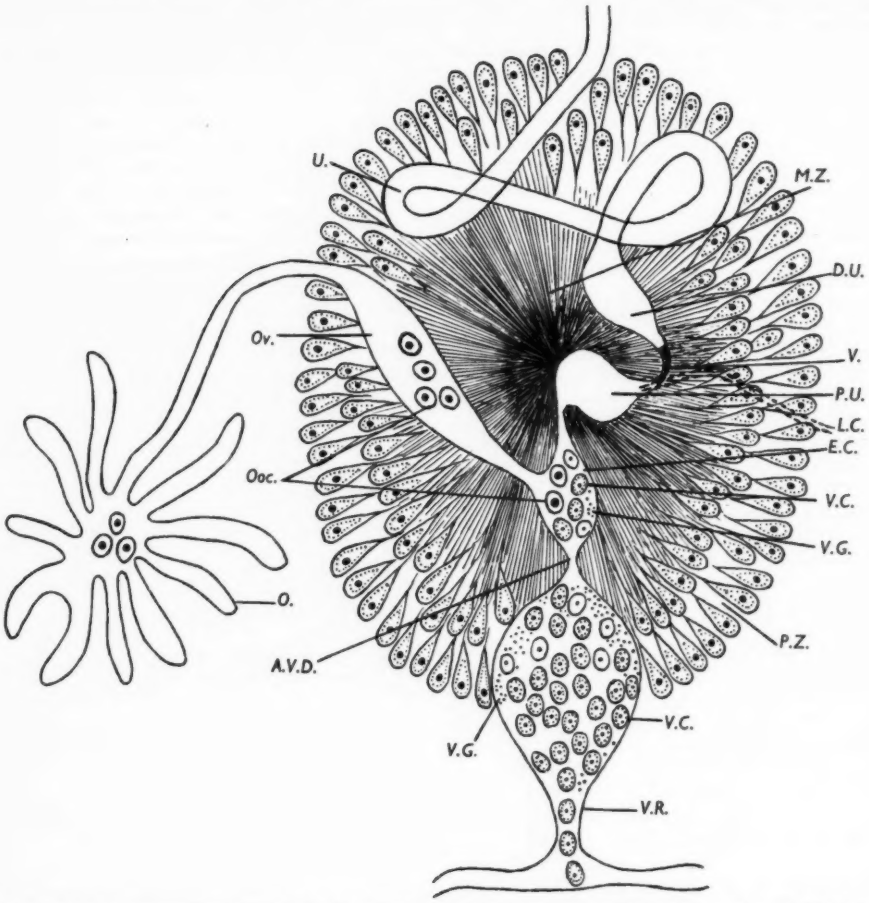
The two transverse vitelline ducts run to the midline of the body of the animal and open into a large cone-shaped structure—the vitelline reservoir (Text-fig. 1, *V.R.*). The reservoir begins at the posterior end of the shell gland and is continued deep into the tissues of the latter. Immediately after the vitelline cells (*V.C.*) enter this chamber they begin to discharge vitelline granules (*V.G.*). That the vitelline cells are stimulated to discharge their granules when they come into contact with, or into the vicinity of, the shell gland suggests that the secretion of this gland plays a double role in shell formation, being responsible for the discharge of the vitelline granules as well as for the production of the hyaline secretion which takes part in the formation of the shell.

From the anterior side of the vitelline reservoir a narrow duct (Text-fig. 1, *A.V.D.*) originates; this duct runs in an anterior direction for a short distance and opens into a wide elliptical chamber situated in the central region of the shell gland (Text-fig. 1; Pl. I, figs. 3 and 4). According to both Schubmann (1905) and Stephenson (1947) neither an ootype nor a receptaculum seminis is present in *F. hepatica*. The elliptical chamber was described for the first time by the present writer (Yosufzai, 1952*a*). Into this chamber the vitelline granules and vitelline cells enter through the anterior mid-vitelline duct (Text-fig. 1; Pl. I, figs. 3 and 4). The oviduct (*Ov.*) runs deep into the shell gland (Pl. II, fig. 5), joins the elliptical chamber and discharges eggs into the latter. The actual connexion between the elliptical chamber and the oviduct was not observed, but the examination of serial sections and the presence of eggs within the chamber leaves little doubt that the oviduct opens into this region. There is a complete absence of sperms within the elliptical chamber. The chamber narrows towards its anterior end, where it almost reaches the centre of the shell gland, and is continued as a narrow duct which joins the proximal part of the uterus. In section, this narrow part appears as a very small space (Pl. II, fig. 6) and probably has been confused by other workers with the opening of Laurer's canal.

When the eggs pass out of the elliptical chamber, the egg-shell is fully formed (Pl. II, fig. 7) and appears as a hyaline structure in silver preparations; the shell has a marked affinity for eosin.

The uterus joins the elliptical chamber. It is divided into a proximal part and a distal part separated by a narrow region or valve (Text-fig. 1, *V.*). Eggs with fully formed shells are present in both the proximal and distal parts of the uterus. The eggs are fertilized in the part of the distal uterus adjacent to the uterine valve and therefore after the shell has been formed; this region contains a huge accumulation of spermatozoa. Spermatozoa were frequently observed in process of penetrating the shell. Laurer's canal (*L.C.*) arises from a minute pore on the dorsal body wall and runs ventrally into the shell gland. The author, however, failed to observe the connexion between the canal and the uterus. There is no trace of an opening from the canal into the oviduct.

The egg-shell of the liver fluke is formed by the hyaline secretion of the shell gland and is later temporarily reinforced by granules liberated from the vitelline cells which surround the eggs as they travel towards the uterus. The vitelline granules ultimately serve as nutriment for the embryo. Additional evidence in support of the author's interpretations was produced by his study of silver preparations, where the eggs were often torn and an inner granular layer was visible inside the outer shell or egg-capsule (Pl. II, fig. 8). The capsule is formed by the secretion of the shell gland, and the inner granular layer is composed of vitelline granules which will ultimately be used up as food by the embryo.



Text-fig. 1. Semi-diagrammatic drawing of the female reproductive organs of *Fasciola hepatica*. *A.V.D.* anterior vitelline duct; *D.U.* distal end of uterus; *E.C.* elliptical chamber; *L.C.* Laurer's canal; *M.Z.* medullary zone of shell gland; *O.* ovary; *Ooc.* oocyte; *Ov.* oviduct; *P.U.* proximal uterus; *P.Z.* peripheral zone of the shell gland; *U.* uterus; *V.* valve; *V.C.* vitelline cell; *V.G.* vitelline granule; *V.R.* vitelline reservoir.

DISCUSSION

Three views have been presented regarding egg-shell formation in trematodes. The classical contention that the shell is formed from the secretion of the shell gland is held by Blumberg (1871), Sommer (1880), Looss (1885), and Schubmann (1905). The more modern conception that the egg-shell of trematodes originates from the globules or granules present in the vitelline cells is supported by Stiles (1894), Leuckart (1886–1901), Henneguy (1902, 1906), Goldschmidt (1909), Augustine (1929), Cable (1931), Taylor (1934), Anderson (1935), Kouri *et al.* (1936), Ujiie (1936*a*, *b*), Kouri & Nauss (1938), Rees (1939), Markell (1943), Stephenson (1947), Willey & Koulish (1950), Willmott (1950) and Smyth (1951). The third view is that the thin outer capsule of the shell is formed from the secretion of the shell-gland and that this is reinforced by the granules of the vitelline cells. This view is held by Tyzzer (1918) and Dawes (1940), and would appear to receive support from the work of Yumoto (1936).

In *F. hepatica* the presence of hyaline material between the protoplasmic strands of the shell gland shows conclusively that this structure produces a secretion as previously reported by Sommer (1880), Macé (1882) and Dawes (1940). The present writer

believes that the egg-shell is formed from the hyaline secretion of the shell gland as claimed by Blumberg (1871), Sommer (1880), Looss (1885) and Schubmann (1905).

During the present investigation it was found that the egg-shell is at first extremely hyaline in appearance, but as the egg travels through the uterus, the shell is temporarily reinforced from the inside by vitelline granules which thus afford greater protection for the egg and later provide food for the developing embryo. In the upper portions of the uterus the thin layer of the shell only is present. In general these observations are in agreement with those of Dawes (1940) for *Hexacotyle extensicauda* and Yumoto (1936) for *Clonorchis sinensis*. To quote Dawes: 'the secretion of the shell glands forms a thin film which serves as a basis upon which the secretion of the "yolk cells" is deposited to form the thin rudimentary capsule, seen in sections'. At another place Dawes wrote: 'It would seem, therefore, that the thin capsule is formed from the free secretion which occurs within the ootype and that it is afterwards reinforced by the deposition of materials on its *inner* surface.' Yumoto, however, did not express an opinion concerning the origin of the two layers, and it would seem that Tyzzer was not at all clear on this matter. It appears that Tyzzer observed the external limiting membrane and noted that egg-shells in early stages of formation were smooth externally and irregular internally, while in advanced stages they are darker, smooth and continuous. He also noted that the yolk cells and fertilized ovum of *Metorchis conjunctum* become surrounded by a membrane before the liberation of globules from the vitelline cells. Tyzzer also recorded the deposition of material on the inside of the shell membrane. Goldschmidt (1909), on the other hand, believed that material is deposited from the outside. It is unfortunate that Tyzzer did not give a clear-cut opinion concerning the formation of the shell. Willey & Koulish (1950) stated: 'That Mehlis's gland plays some role in formation of the shell seems indicated by the fact that in some trematodes which lack the gland the egg shells are thin and membranous.' They quote Stunkard (1943) who in *Zoogonoides laevis* reported: 'There is no "shell gland" and the miracidia develop in thin-walled, membranous sacs. . . . The egg membrane is flexible and the shape varies with pressure.'

The present writer believes that, because of the routine methods of technique which they employed, most of the previous workers failed to observe the hyaline secretion of the shell gland.

Recently, Smyth (1951) showed that malachite or methyl green stain the egg-shell and the vitelline granules of trematodes, and he concluded that the vitelline granules give rise to the egg-shell. During the present investigation it was observed that there are other dyes which stain the vitelline granules and the egg-shell, for example eosin. It seems probable that the stains used by Smyth failed to stain the hyaline egg-shell but simply stained the vitelline granules present beneath the shell.

The present author's observations on the histology of the shell gland are in agreement with those of Stephenson, except that the latter failed to note the presence of the hyaline secretion. Ujiie (1936*a*) stated that two types of cells are present in the shell gland of *Echinochasmus japonicus*, but as cells of two kinds have not been recorded as present in the shell gland of any other trematode it is probable that Ujiie has interpreted the small nuclear-like bodies of the protoplasmic strands as a second type of cell.

The writer's observation that vitelline granules are released immediately after the vitelline cells come in contact with the shell gland, are in agreement with those of Henneguy (1906), for *Fasciola hepatica* and of Tyzzer (1918) and Ujiie (1936*a*, *b*) for other trematodes, but does not agree with the findings of Goldschmidt (1909), Murikama (1937), Kouri & Nauss (1938), Stephenson (1947), Willmott (1950) and Smyth (1951).

A glance at the literature of the subject shows that there is considerable disagreement as to the region of the female genital ducts in which the vitelline granules are released and in which the shell is formed. Stephenson stated that—'the vitelline cells are churned about in the uterus, where they become mixed with ova, spermatozoa, and recently formed shelled eggs. If no spermatozoa are present, there is generally only a slight discharge of granules. This slight discharge may be due to the more prolonged action of Mehlis's gland secretion, or more probably to mechanical forces.' At another place the same author wrote: 'In some individuals (see, for example, Pl. II, fig. 2), liberation of granules begins immediately after the vitelline cells pass the uterine valve. It seems likely that this is due to mechanical forces which would act on the cells as they are squeezed through the valve.' Further, he stated that 'present work entirely confirms Henneguy's (1906–7) observation that no visible secretion is produced'. In view of Stephenson's contradictory statements and of the findings of Henneguy (1906) and the present author, it is clear that Stephenson's theory that mechanical force is responsible for the liberation of the vitelline granules and his observations regarding the place of discharge of the granules cannot be accepted.

The author observed eggs with fully formed shells in the proximal (Stephenson's central chamber of Mehlis's gland) and in the distal portions of the uterus; Stephenson (1947), however, reported the absence from the central chamber of ova provided with shells. Recently, Willmott (1950) claimed that the shell is formed in the central chamber of *Giganto-*

cotyle bathycotyle. The presence of fully formed egg-shells in the proximal part of the uterus disproves Stephenson's argument that liberation of granules takes place in the more distal uterine coil only.

In view of the present findings the writer believes that the suggestion of Goldschmidt (1909), Kouri & Nauss (1938) and of Smyth (1951) to change the term shell gland to Mehlis's gland is not desirable. The secretion of the shell gland is used in the formation of the shell and hence the term shell gland indicates the function of that structure.

The elliptical chamber described by the author (Yosufzai, 1952*a*) is not identical with Stephenson's 'central chamber of Mehlis's gland'; the latter takes its origin from the junction of the anterior vitelline duct and oviduct. The elliptical chamber originates from the anterior mid-vitelline duct and extends to the middle of the shell gland. Most of the previous investigators based their conclusions on reconstructions of their sections. Kouri & Nauss gave a figure of a section containing a portion of the elliptical chamber, but labelled it Laurer's canal; their figure 3 (p. 304) exactly resembles the author's figure (Pl. II, fig. 6). It is fortunate that the writer obtained a section showing both the anterior vitelline duct and the elliptical chamber thus clearly demonstrating the relationship between these two structures.

The writer has shown that oocytes, vitelline granules and vitelline cells enter and are mixed together in the elliptical chamber, and here the secretion of the shell gland is discharged and the egg-shell is formed. Stephenson believes that movement of the spermatozoa may be responsible for 'relative constancy, and for the positioning, of the cells within an egg'. The present investigation shows that sperms are not present in the elliptical chamber and that the egg-shell is laid down while the ova are within the chamber. The author, therefore, considers that the arrangement of the cellular constituents within the egg is determined in the elliptical chamber which performs a similar function to the ootype of other trematodes.

SUMMARY

1. The shell gland of *Fasciola hepatica* produces an extremely hyaline secretion. The egg-shell is formed from the hyaline secretion.

2. As the eggs travel through the uterus, the shell is temporarily reinforced from the inside by vitelline granules. The vitelline granules are released when the vitelline cells enter the vitelline reservoir.

3. Views regarding the nomenclature of the shell gland are discussed.

4. The presence of an elliptical chamber in the shell gland is described. In this chamber, oocytes, vitelline granules and vitelline cells are mixed together, and here the secretion of the shell gland is released and the egg-shell is formed.

5. The arrangement of the cellular constituents within the egg is effected by the elliptical chamber.

The writer wishes to express his gratitude to Prof. R. A. R. Gresson, for suggesting this problem and for guidance during the course of the investigation. His thanks are also due to Mr W. Ferguson who took the photomicrographs.

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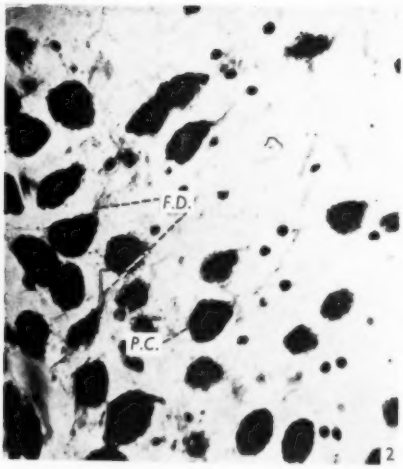

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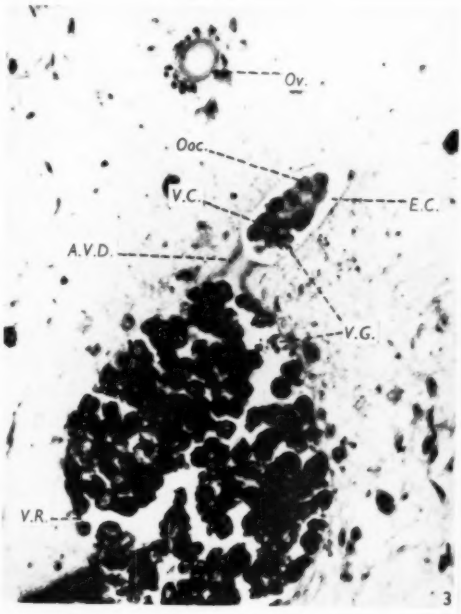

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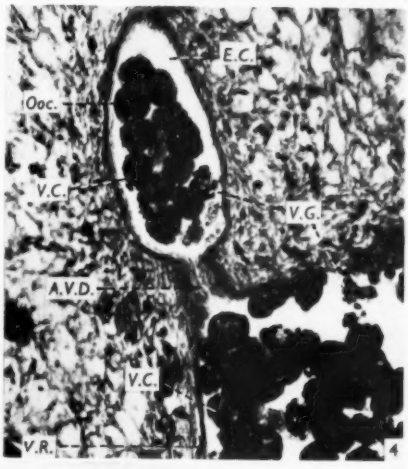

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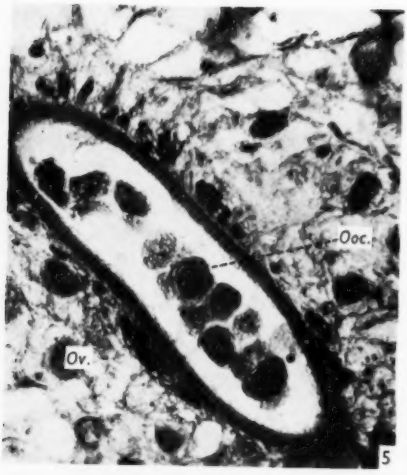

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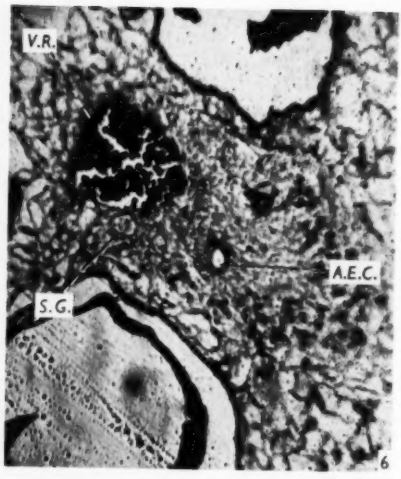

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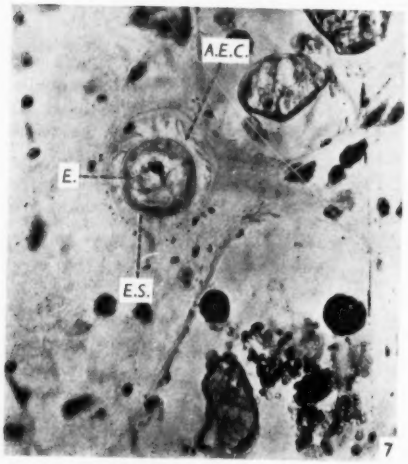

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Photomicrograph of shell gland. To show medullary zone with radiating protoplasmic strands converging towards the centre. The hyaline secretion is present between the strands. Cajal.

Fig. 2. Photomicrograph of part of the peripheral zone of the shell gland. To show cells with funnel-like ducts. Cajal.

Fig. 3. Photomicrograph showing the vitelline reservoir, the anterior vitelline duct and the elliptical chamber. Bouin's picro-formol.

Fig. 4. Photomicrograph of vitelline reservoir, anterior vitelline duct and the elliptical chamber. Bouin's picro-formol.

PLATE II

Fig. 5. Photomicrograph of the part of the oviduct within the shell gland. Bouin's picro-formol.

Fig. 6. Photomicrograph to show the anterior part of the elliptical chamber in the central region of the shell gland. Bouin's picro-formol.

Fig. 7. Photomicrograph of egg with a newly formed egg-shell. Da Fano.

Fig. 8. Photomicrograph of eggs with torn shells showing the outer capsule and the inner granular layer. Da Fano.

KEY TO PLATES

A.E.C.	Anterior opening of elliptical chamber	*Ooc.*	Oocyte
A.V.D.	Anterior vitelline duct	*Ov.*	Oviduct
E.	Egg	*P.C.*	Peripheral cells
E.C.	Elliptical chamber	*P.S.*	Protoplasmic strand
E.Ca.	Egg-capsule	*S.G.*	Shell gland
E.S.	Egg-shell	*U.*	Uterus
F.D.	Funnel-like duct	*V.C.*	Vitelline cells
H.S.	Hyaline secretion	*V.G.*	Vitelline granule
I.V.G.	Inner layer of vitelline granules	*V.R.*	Vitelline reservoir
M.Z.	Medullary zone of shell gland		

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THE REPRODUCTIVE HABITS AND SEX-DETERMINATION OF THE NEMATODE *RHABDITIS PELLIO* BÜTSCHLI, WITH A NOTE ON ITS TAXONOMY AND NOMENCLATURE

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INTRODUCTION

The nematode group of worms contains numerous parasitic and free-living species occurring abundantly in marine, fresh-water and terrestrial habitats. The reproductive habits of these worms show great variation. In the marine forms they are usually dioecious and the sexes occur in equal proportions; but various workers (e.g. Maupas, 1900; Potts, 1910) have shown that hermaphroditism is not uncommon in terrestrial forms, and that females are often much commoner than males. In some species males are absent altogether.

It is in this connexion that the reproductive habits of *Rhabditis pellio* Bütschli are of great interest. The main work on this nematode was published by Johnson in 1913. He obtained the nematodes from the nephridia and coelom of earthworms where they remain in a larval stage until the death of the host. Development, once started, continues rapidly, and under favourable conditions generations of the nematodes will succeed one another at the rate of about one a week. Johnson found that both males and females of the nematode could be obtained from rotting earthworms. The females apparently were capable of reproducing parthenogenetically* for an indefinite number of generations, and copulation with males was never observed in any of the cultures into which they were introduced. The proportion of males to females was always small (about 1 : 5) and it seemed questionable whether they had any function.

This nematode might be considered to represent an evolutionary stage between dioecious species and those whose males had become so unnecessary that they had been discarded altogether; but there is an important discrepancy between the results obtained from cultures *in vitro* and the situation found in the natural state, in that although male nematodes were invariably present in rotting earthworms they very rarely appeared in laboratory-bred cultures.

* 'Parthenogenetic' throughout this account is intended to imply only reproduction without fertilization by a male. Difficulties concerning the meaning of 'true parthenogenesis' and 'hermaphroditism' will thus be avoided.

The investigation was greatly hampered by the lack of any reliable culture technique. Johnson's cultures frequently completely died out without any observable cause, and he often made deductions from cultures in which only 20–30% of the nematodes survived. A later worker, Otter (1933) found it impossible to rear a single F_2 generation.

It was with these problems in mind that I decided, two years ago, to re-investigate the behaviour of this nematode. My work has been concerned with answering the following questions:

(1) Can a reliable method of culture be devised for *R. pellio*?

(2) Why is it that though males are invariably found in rotting earthworms they so rarely appear in cultures *in vitro*?

(3) Do the males have any function, i.e. is reproduction ever bisexual?

(4) How is the curious sex ratio to be accounted for?

(5) Are two species being confused under the name *R. pellio* Bütschli, the one bisexual and the other purely parthenogenetic?

(6) A nematode has recently been obtained from earthworms and named *R. terrestris* Stephenson. Its characteristics seem remarkably similar to those of *R. pellio* Bütschli. Is it in fact the same species?

It will be convenient to describe my work in six sections corresponding to the questions above.

(1) METHOD OF CULTURE

The results obtained by previous workers and by myself, from rearing *R. pellio* in laboratory conditions, have shown that great variety of size and behaviour of the worm may be produced by differences of culture medium. The nematodes are of microscopic size and may often be reared in watch-glasses in highly artificial media (e.g. early workers succeeded in culturing them in peptone solution) but the results are invariably very poor, the nematodes reared being much undersized and producing few progeny. Recently, Dougherty & Calhoun (1948) have developed a technique for growing the worms on carefully selected strains of bacteria. As my

investigation was primarily concerned with problems of the worms behaviour under natural conditions, it was essential that a culture medium be used as much like the normal environment of the nematode as possible. Accordingly, I attempted to use an 'earthworm broth', as used by Johnson, made by boiling earthworms in water for 2 hr. and inoculating the extract so formed with bacteria from a rotting earthworm. This medium when successful produces nematodes of size and appearance identical with those emerging from rotting earthworms; but it had the disadvantage that the results were unpredictable. Some cultures thrived splendidly whilst others died off.

if concentration of the medium itself was the cause of death. An earthworm was opened by a mid-dorsal incision and the gut removed. A long section of the trunk was then divided up as follows:

A	*B*	*C*	*D*	→Anterior
Aa	*Ba*	*Ca*	*Da*	

The pieces *Aa*, *B*, *Ca*, *D* were each placed intact in separate watch-glasses. Very little water was added and the cultures rapidly became a semi-solid mass of putrefaction. From the pieces *A*, *Ba*, *C* and *Da*, only the nephridia were kept and these were placed

Table 1

Males	Females	Larvae			Males	Females	Larvae	
1	20	13	*A*	*Aa*	1	15	1	
	13	14	*Ba*	*B*	1	23	3	
	12	12	*C*	*Ca*		34	3	
	6	3	*Da*	*D*		9	1	
1	51	42 =	Total 94		2	81	8 =	Total 91

The failures appeared to be of two types:

(*a*) This was most noticeable when it was attempted to rear an exact (usually small) number of larvae. It was often found that this number gradually diminished and only a few of the larvae reached maturity.

One explanation of this loss was found from observation of the behaviour of the young larvae. They often have a very strong tendency to move to the extreme periphery of the culture (probably connected with a taxis for leaving dead earthworms in the natural state) and are very apt to get caught in the deposit of salts formed there immediately any evaporation of the medium takes place. This inevitably leads to death through desiccation.

This can be prevented by rearing the cultures in a highly saturated atmosphere and examining them frequently for the first signs of larvae becoming trapped.

(*b*) The more serious type of culture failure was quite different. On one day a culture would consist of fully active, apparently perfectly healthy nematodes, and on the next day every single worm of whatever age would be stretched out perfectly straight and motionless on the bottom of the watch-glass, not one of the worms giving the least indication of life. These defective cultures were frequently accompanied by an unusual cloudiness of the medium and the appearance of a thin scum over the top.

It was noticed that losses of this type occurred much more frequently in cultures of highly concentrated medium than in those where it was more dilute. The following experiment was made to test in watch-glasses with plenty of water, resulting in a very dilute culture medium.

After 3 days the nematodes present in the cultures were counted, and the results are given in Table 1. (None of the sets of figures presented in this account has been subjected to rigorous statistical analysis. Though this might be desirable theoretically, it has been felt that in practice the arguments based on the figures given are sufficiently justified by these figures taken at their face value.)

All the worms looked healthy and the results show that high concentration of medium itself is not detrimental to the nematodes. (The higher proportion of females to larvae in the concentrated media could be accounted for in several ways, e.g. (i) the higher concentration of putrefaction caused more larvae to develop, (ii) larvae must inevitably have been overlooked in the opaque pieces of flesh in the concentrated cultures, (iii) some nematodes are bound to be lost when the nephridia are scraped off the body wall for the dilute cultures.)

However, although a concentrated medium may not in itself be harmful, it may favour the growth of micro-organisms which are harmful; so perhaps this difficulty could be avoided by rearing the nematodes in more dilute cultures. Unfortunately, this is not a satisfactory solution. Dilution of the cultures to an extent where they are 'safe' for the preservation of life quickly leads to undersized nematodes, and if carried too far considerably delays growth, so that it is doubtful how far we are entitled to make deductions from these cultures about the natural life of the nematodes.

The key to the problem was found in an experiment on one culture which had apparently just died. The day previously it had appeared perfectly healthy, but overnight the worms had lost all signs of life. Instead of discarding the culture as usual, most of the medium was carefully decanted off and replaced with distilled water. Within 1 hr. the worms had slowly begun to move; a little fresh culture medium was given, and by the end of the day the whole culture was perfectly normal again. It appeared none the worse for this lapse and did not apparently differ from similar control cultures which had not so suffered. Four days later it was still healthy but the worms had increased to such vast numbers that only a few were kept and the rest destroyed. Three weeks later descendants of this culture were still thriving. I have not determined how long after the appearance of the first symptoms revival is possible, but if the cultures are examined daily, and treated by the above method if necessary, it is reasonably certain that a given culture will thrive indefinitely. I have changed the media in this way several times in cultures which would previously have been discarded as 'dead' and since employing this technique have not had a single failure.

The differences between closely related species of nematodes often being slight, considerable care has been taken to ensure that *R. pellio* Bütschli is the only nematode being used. The copulatory bursa of the males has been examined frequently during the experiments and has always been found to correspond exactly with the description given in Johnson's paper. There is no such valuable criterion for the females, but measurements of the proportions of the sizes of the body organs correspond with those given by him; also, of course, there can be no doubt that the females are the same species in those cultures where I have found undoubted bisexual reproduction to take place. Usually I have obtained nematodes from the nephridia only of earthworms, and in these cultures nothing but *R. pellio* Bütschli has ever been observed. Occasionally, in cultures begun from nephridia and body wall a few other forms of nematodes have appeared. *R. pellio* Bütschli has been obtained from earthworms of the genera *Lumbricus* and *Allolobophora*, but is much commoner in the former, and *Allolobophora* earthworms frequently appear to be quite uninfected.

The culture procedure which I find gives very good results may be summarized as follows:

(i) Obtain the nematodes from the nephridia only, of species of *Lumbricus* earthworms. The earthworms are opened, the gut removed and the nephridia scraped in bulk from the body wall and placed in covered watch-glasses with a little tap water.

(ii) For subsequent subcultures grow the nematodes in Johnson's 'earthworm broth'. The medium should be kept fairly strong, the best test of this being whether the nematodes at each stage of development compare favourably in size with those in rotting earthworms.

(iii) A fresh supply of earthworm broth should be made up every 3 or 4 days. The cultures should be examined daily, and at the first signs of large numbers of straight motionless worms appearing the medium should be changed for the freshest medium in stock.

(iv) In cultures where it is desired that an exact number of larvae should reach maturity a careful watch should be kept on the periphery of the culture for worms being trapped there (a hanging-drop technique might work better here but I have not tried it).

(v) No difference has been noted in cultures reared in warm weather or cool weather, light or darkness, sealed watch-glasses or a free supply of air so these factors may be ignored.

Table 2

20. iii. 50	Parent culture	Several females	3 F.	3 F., 1 M.
	No. of F_1 eggs reared	10–15	20–40	50–100
30. iii. 50	No. of F_1 adults	11 F.	30 F.	97 F.
	No. of F_2 eggs reared	1	14	10
8. iv. 50	No. of F_2 adults	1 F.	14 F.	10 F.
	No. of F_3 eggs reared	—	—	Many
	No. of F_3 adults	—	—	Many F.
	No. of F_4 eggs reared	—	—	Many
20. iv. 50	No. of F_4 adults	—	—	Many F.

F. = females; M. = males.

(2) THE PRODUCTION OF MALES

There is no doubt that under certain conditions generation after generation of these nematodes may occur without the production of any males. Johnson mentions several instances of this, and the above culture series of mine illustrate the same point (Table 2).

It is clear from these results that the production of males cannot be (*a*) an inevitable result of rearing from parent cultures containing only females, or males and females, since both cases have been tried and males have not turned up in the subsequent generations of either; or (*b*) dependent on the total number of worms present in a given culture (as is believed to happen with some nematodes), since various numbers of worms ranging from one to several hundreds in cultures of similar volume were tried.

It is notable that in Johnson's experiments he obtained males in filial generations only when males were present in the parent generation. I have reared numerous cultures of worms from parent cultures containing females only or females plus males in an attempt to find whether this was invariably true. These parent cultures contained different numbers of female parents and different combinations of males and females. In some cases the males and females were seen in copulation when the culture was made up. The results are summarized in Table 3.

Table 3

(*a*) No. of parental cultures containing only females*	16†
(*b*) No. of cultures of progeny from (*a*) which contained only females	16
(*c*) No. of cultures of progeny from (*a*) which contained males and females	0
(i) No. of parental cultures containing males and females‡	67
(ii) No. of cultures of progeny from (i) which contained only females	35
(iii) No. of cultures of progeny from (i) which contained males and females	32

* 'Only females' implies the females have had no contact with males since early larval stages.

† Total number of females over 100.

‡ 'Males and females' implies that the females have had opportunity for copulation even though the males may not be present during egg laying.

It will be seen that though males never appeared in cultures containing no males in the parent generation, they appeared frequently where parental males were present. Since I have often observed copulation taking place, and (as will be seen in a later experiment) when cultures are made up only from copulating worms the proportion of filial cultures containing males is much higher than the ratio 32 : 35 shown above, it may be that males are produced only from eggs fertilized by sperm from male parents.

This explains why most of Johnson's cultures produced only females, since he concentrated on rearing larvae from cultures containing only females, and when he introduced males presumably copulation did not take place.

Similar results have been found recently in other nematodes. Dougherty & Nigon (1949) found with *Rhabditis elegans* and *R. briggsae* that when breeding from parthenogenetic females fertilized by males, up to 49% male offsprings were produced, but only about three males to 5000 females occurred in the progeny of females alone. Hyman (1951), summarizing the work on Rhabitoids, states that often the 'males, though anatomically normal, have lost the sexual instinct and usually will not copulate; when they do so, the offspring contain many more males than is usual for the species'.

I have not observed any males in cultures reared from isolated females, but I have not made counts as large as 5000, and I may have failed to detect very small proportions of males; but they certainly do not occur in such cultures with a greater frequency than 1·0% of the progeny.

(3) THE FUNCTION OF THE MALES

It will be clear from the above that bisexual reproduction frequently does take place. Copulation has often been observed and is necessary for the production of a further generation of males. Sometimes, however, bisexual reproduction is not only a possibility but is obligatory if the females are to lay viable eggs. On several occasions the progeny of a single isolated female have grown to sexual maturity but have failed to deposit eggs. They appear of good size, perfectly healthy, and numerous ova can be seen within them. I have sometimes kept them 2 or 3 weeks after females of similar age in other cultures have begun producing eggs, but no eggs are laid.

I have found that in such females reproduction is obligatorily bisexual. A culture was chosen where only one parent female was present and all her offspring were females (there had been a parent male present but he was removed whilst the offspring were eggs or very young larvae). Twenty-nine of the young females were divided into eight cultures. To four of these cultures males were added, and the other four contained only females. The males added to two of the cultures were a day or two older than those added to the other two (Table 4).

It was hoped that cultures 1–4 would produce males and females, and that cultures 5–8 would produce only females. Three days later only cultures 3 and 4 had produced any young, and these two cultures contained masses of eggs and young larvae. The females in the remaining six cultures looked perfectly healthy, of natural size and reasonably active. However, although their ovaries appeared

Table 4

Culture no.	No. of males	No. of females
1	6 (rather old)	3
2	6 (rather old)	3
3	7 (young)	4
4	5 (young)	3
5	0	4
6	0	4
7	0	3
8	0	5

packed with eggs only a few were to be seen in the oviduct region, and these appeared abnormal and lacked the clear-cut outlines of normal eggs. A very few eggs of abnormal appearance had been laid.

It was suspected that the females were unable to reproduce without fertilization by a male, and that this had occurred in cultures 3 and 4 but the males were too old for the purpose in cultures 1 and 2. In order to test this, four young males were added to each of cultures 5 and 6. The result was startling; in about 6 hr. numerous developing eggs had been laid in both cultures, one female in each was seen in copulation, and three out of the remaining six females had numerous healthy eggs in the oviduct.

Ten days later cultures 1, 2, 7 and 8 had produced no viable eggs, whilst cultures 3–6 had produced hundreds of young which proved in all four cases to be of both sexes (Table 5).

Table 5

Culture no.	Males	Females	Result
1	6 rather old	3	No young
2	6 rather old	3	No young
3	7 young	4	Many M.+F.
4	5 young	3	Many M.+F.
5	4 young added later	4	Many M.+F.
6	4 young added later	4	Many M.+F.
7	0	3	No young
8	0	5	No young

M. = male; F. = female.

It may be objected that it was something transmitted in the medium with the males that stimulated egg laying. However this seems most unlikely in view of the facts that:

(1) Copulation was actually observed in the cultures in question.

(2) Isolation of the males used had necessitated considerable dilution of their culture medium with water, and only a very small amount of this was introduced with them.

(3) In other experiments I have replaced the culture medium of non-reproducing females with that of freely reproducing females which had not copulated. Such a change has never stimulated egg laying.

(4) On one occasion nine cultures were made under similar conditions from the same earthworm. Six of these cultures each contained an isolated female and produced no progeny: the other three contained males and females and produced numerous offspring.

It appears certain therefore that obligatorily bisexually reproducing generations occur in this nematode species.

(4) THE SEX RATIO

I have grown several cultures with the object of finding the sex ratio in the mixed offspring resulting from females fertilized by males. In order to avoid confusing the slowest growing (or youngest) larvae of the first filial generation with the fastest growing (or oldest) of the next, only the eggs laid by the parent female within a limited length of time were used; the resulting larvae then all become sexually differentiated within a fairly short time.

In an early experiment several females were isolated from a culture which contained males; the females were distributed one per watch-glass, and destroyed after they had laid eggs for 24 hr. In five cases both males and females occurred in the progeny (Table 6).

Table 6

	Males	Females
1	12	15
2	25	22
3	11	17*
4	22	12
5	13	32*
Total	83	98

* Contained some rather young larvae of which it was difficult to be certain of the sex.

Although the totals might be said to approach a 1 : 1 ratio the results appeared to me to be unsatisfactory on account of the very great differences in the ratios found in the separate cultures.

I therefore repeated the experiment with the following differences.

(1) Only females which were seen in copulation were used—this avoided having numerous 'useless' cultures.

(2) They were allowed to lay eggs for a longer time. Where necessary, to avoid any risk of confusing two generations, the F_1 were counted in two stages. At the first count the culture was distributed in small drops on a slide and the contents of each drop counted. The criterion for male was taken to be the possession of a copulatory bursa, and for a female the possession of a vagina or rudiment of that organ. Those nematodes in which neither could be detected were returned to the culture and counted at a later date. This inevitably involves a small loss of nematodes, but this loss has been recorded and will be seen to be on a scale which does not affect the

conclusions drawn: it should be noted, however, that these lost nematodes are likely to have the sex proportions found in the second count rather than in the total (Table 7).

by the male is the cause of the results. The only certain method would seem to be to rear the nematodes from eggs which are seen to have been fertilized by spermatozoa.

Table 7

No. of culture	Date when set up (Sept. 1951)	Date when parents removed (Sept. 1951)	First count				Second count			Total		
			Date (Sept. 1951)	M.	F.	Undetermined	Date (Sept. 1951)	M.	F.	M.	F.	Lost
1	20*	23	26	2	62	5	28	0	4	2	66	1
2	21	24	27	3	37	32	28	13	13	16	50	6
3	21	24	27	1	62	61	29	22	27	23	89	12
4	21	23	27	28	71	0	—	—	—	28	71	0
5	21	23	27	75	98	2	—	—	—	75	98	2
6	21	23	27	12	38	0	—	—	—	12	38	0
7	21	24	28	1	73	0	—	—	—	1	73	0
8	22	24	28	2	93	0	—	—	—	2	93	0
9	21	24	27	4	66	39	29	2	34	6	100	3
10	22†	24	27	0	38	4	29	0	4	0	42	0
11	22‡	24	28	6	68	1	—	—	—	6	68	1
12	21	23	27	2	48	0	—	—	—	2	48	0

M. = male; F. = female.

* Male and female broke apart as culture made up. Only female used.
† Male and female broke apart as culture made up. Both used.
‡ 23 Sept. 1951, male and female again *in copulo* though eggs already laid.

It will be seen that though the ratio of males to females is still highly variable it is always less than 1 : 1. These results might be explained by the following hypothesis.

(1) The ratio never exceeds a 1M. : 1F. value because sex determination is of the *XO* (or *XY*) and *XX* type where *XO* is male and *XX* female.

(2) The proportion of males often falls so far short of this value because innumerable females can be produced parthenogenetically.

In support of this hypothesis are the facts

(*a*) Chitwood & Chitwood (1950) give the heterochromosome constitution of *R. pellio* Bütschli as *XO, XX*.

(*b*) Johnson (1913) mentions a generation consisting of 36M. : 31F. as though this were the highest ratio he obtained. This could be considered to approximate to a 1 : 1 ratio.

(*c*) The proportions of the sexes obtained from rotting earthworm always give a low value for males. Johnson (1913) quotes 1M. : 5F. as an average figure and I have obtained values ranging from 1M. : 40F. to 1M. : 4F.

(*d*) In my first sex-ratio experiment four of the results conform with this hypothesis.

Against this hypothesis are the facts

(*a*) In my first sex-ratio experiment one of the results showed a ratio 22M. : 12F.

(*b*) So long as we suspect that the females are able to reproduce without male fertilization, we can never be certain of the extent to which fertilization

On the whole, however, the evidence seems to me to be much more strongly in favour of this hypothesis than against it.

(5) DOES *RHABDITIS PELLIO* BÜTSCHLI COMPRISE ONE OR MORE SPECIES?

The problem may be stated this way. There are obtained from rotting earthworm nephridia three forms of nematode.

(1) Males—all anatomically identical down to the finest details of the reproductive organs.
(2) Parthenogenetic females. } Anatomically in-
(3) Non-parthenogenetic females. } distinguishable from each other.

How many species does this represent?

Considered logically, either the parthenogenetic females and non-parthenogenetic females belong to different species or to the same one. If they belong to the same species then no question arises as to the status of the males, but if two separate species are represented, two types of male may be present corresponding to the two types of female.

The problem would be most simply solved, therefore, if it could be shown that the parthenogenetic females and non-parthenogenetic females belong to the same species. This could most conveniently be done by showing that one form can give rise to the other. Unfortunately, I have no certain proof that this has occurred in my cultures, but the following

considerations would make it appear extremely likely.

(1) On two occasions single females have given rise to families consisting solely of non-parthenogenetic females (forty-two in one case and about thirty in the other). It is most unlikely that an obligatorily bisexually reproducing species would produce monosexual families of this size.

(2) In the experiment mentioned previously where non-parthenogenetic females are known to have been in cultures with males, the progeny included large numbers of males. Hence it would seem probable that the entirely non-parthenogenetic-female families which have occurred were produced parthenogenetically.

Stephenson describes the lips as being three in number, each with a single papilla. In view of all the similarities this slight difference of description hardly seems sufficient grounds for establishing a new species. It is noteworthy that Stephenson does not include Johnson's 1913 paper in his bibliography; it seems likely that he was unknowingly working with *R. pellio* Bütschli and has mistakenly renamed it under the impression that it is a new discovery.

Johnson's name for this nematode originated from the fact that previously two species had been confused under the name *R. pellio*. For one he proposed the name *R. pellio* Schneider and for the other *R. pellio* Bütschli. Clearly this was incorrect since specific difference should be indicated in the second

Table 8

	R. pellio Butschli as described by Johnson (1913)		*R. terrestris* Stephenson as described by Stephenson (1942)
	Normal* (μ)	Grown on peptone (μ)	Range under all conditions (μ)
Female:			
Length	2000	1238	930–2580
Oesophagus	226	248	196–256
Tail	150	143	116–184
Vulva (from anterior end)	1019	638	49–58% (= % of total length)
Diameter at vulva	143	86	52–114 (or 136 including lips)
Buccal tube	24	—	28–34
Male:			
Length	1700	900	626–1330 (males rarely found)
Oesophagus	209	—	192–208
Tail	72	64	36–56
Diameter	104	50	42–70
Buccal tube	23	—	24–30

* 'Normal' = usually the largest individuals found.

(6) *RHABDITIS TERRESTRIS* STEPHENSON

Stephenson (1942*a*, 1942*b*, 1944, 1945) has recently published some papers in which he describes variations in form and size of a nematode under varying culture conditions. He has named this nematode *R. terrestris*. However, it has numerous similarities with *R. pellio* Bütschli as described by Johnson (1913). Both nematodes are obtained from earthworms. The cuticle, buccal tube, oesophagus and male and female reproductive organs are given similar descriptions by both authors. The drawings given by both authors appear to be of identical nematodes. The anatomical measurements in the two cases are given in Table 8.

The only difference I can find is in the description of the lips of the worms. Johnson says 'The mouth is bordered by three lips, one dorsal and two ventral, each of which is divided by a shallow groove into two lobes. Each lobe bears a pair of fine short papillae.'

and not the third name. As '*R. pellio*' is often mentioned in the subsequent literature without the third name, confusion inevitably arises as to which species is being referred to. It would seem desirable to rename one of the species. Chitwood lists *R. maupasi* Caullery & Seurat 1919 as synonymous with *R. pellio* Bütschli so possibly this name could be used. But the whole genus *Rhabditis* is in such confusion at present that great care is necessary in the use of nomenclature. The nematodes show great variation under different culture conditions and the differences between the species are often very slight indeed. Goodey (1951) says, 'The genus (*Rhabditis*) is represented by a large number of species and at the present time needs monographing and systematizing.' In fact he considers *R. terrestris* Stephenson as probably synonymous with *R. aspera* and *R. lucanii*.

It is only possible, therefore, to state that the nematodes discussed in this account conform to the

description of *R. pellio* Bütschli as given by Johnson 1913. An alternative name would be desirable, but should be deferred for a careful revision of the whole genus *Rhabditis*.

SUMMARY

1. The nematode *Rhabditis pellio* Bütschli may be cultured indefinitely on earthworm extract.

2. Although the females of this nematode are usually capable of parthenogenetic reproduction, males are produced in the offspring only if copulation has taken place.

3. Occasionally non-parthenogenetic generations appear but these do not represent a separate species.

4. An explanation is given for the sex ratio found under natural conditions and it is suggested that sex-determination works on a chromosomal arrangement of XO = male and XX = female.

5. The nematode named *R. terrestris* Stephenson 1942 is probably the same as that named *R. pellio* Bütschli by Johnson 1913.

6. The nomenclature of *R. pellio* Bütschli is discussed.

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HUMAN TRICHOSTRONGYLOSIS AND ITS RELATIONSHIP TO ANCYLOSTOMIASIS IN SOUTHERN IRAQ, WITH COMMENTS ON WORLD INCIDENCE

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(With 3 Figures in the Text)

INTRODUCTION

Species of the genus *Trichostrongylus*, though common in all parts of the world as parasites of ruminants and rodents, have been less frequently found in man than other intestinal nematodes. Stoll (1947) estimated that the several species of this genus constitute a worm-burden for 5·5 million human beings, mainly natives of Asia, as compared with 35 millions infested with *Strongyloides stercoralis*, 209 millions with *Enterobius vermicularis*, 355 millions with *Trichuris trichiura*, 457 millions with hookworm and 644 millions with *Ascaris lumbricoides* (see Fig. 1).

Within recent years, however, records of human infestation with species of *Trichostrongylus* have been appearing with increasing frequency, especially from India, Russia, Africa and the Far East; and it is becoming apparent that Stoll's estimate was ultra-conservative. Available data indicate that the incidence and distribution of human trichostrongylosis is by no means uniform. Only occasional cases, possibly pseudo-infections, have been reported from Europe and America. On the other hand, Lie Kian Joe (1947) found a 40% incidence among Indonesians in Java, Van Slype (1934) a 10% incidence in the Belgian Congo, Webb (1937) an 8% incidence in Mauritius, Chandler (1926, 1927), Maplestone (1941) and others an incidence ranging from 1 to 25% in different parts of India, and Hunter and his collaborators (1948 to 1951) an incidence ranging from less than 1% to over 82% in different parts of Japan and Korea. Recently, Stewart (1949) added South Persia to the list of endemic areas with a reported 70% incidence among labourers working for the Anglo-Iranian Oil Company.

PRESENT INVESTIGATIONS

In the course of a recent survey of the incidence and distribution of hookworm and other intestinal helminths among the population of Iraq it was found that *Trichostrongylus* was a common parasite of man along the lower course of the Tigris and Euphrates in the area just north of and continuous with that investigated by Stewart.

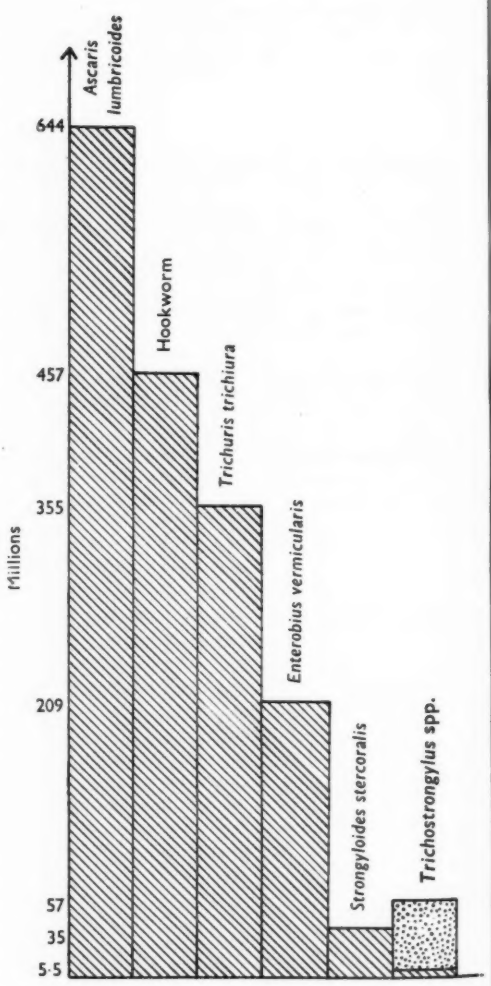

Fig. 1. Number of individuals infected with the principal intestinal helminth parasites. (World totals after Stoll, 1947.)

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The incidence decreases northwards from Abadan past Basrah up the course of the Shatt-al-Arab estuary. Along the humid and palm-fringed banks of the Euphrates upstream from its confluence with the Tigris at Qurnah the incidence continues to fall but less rapidly than along the more arid banks of the Tigris (see Table 1, Figs. 2 and 3). No indigenous cases were found above Kurmah on the Euphrates or above Amarah on the Tigris. (The occurrence of a few cases in Baghdad was no doubt due to the presence of immigrants from the south.)

Inland from the banks of the main rivers human trichostrongylosis hardly occurs at all. At Hillah and at Diwaniyah, which are served by canals taking off from the left bank of the Euphrates at Museiyeb, just above the Hindiyah barrage, no cases were found; but at Kerbala, which is watered by a canal taking off from the right bank at the same point, a small number of parasitized individuals were found. They may, however, have been pilgrims from the southern districts rather than indigenous inhabitants.

This apparently anomalous distribution is strictly correlated with local climatic conditions favourable to the development and survival of the free-living juvenile stages of *Trichostrongylus* which require a high humidity, the protection of shade from the intense tropical sun and the presence of ground vegetation for the migration of the juveniles. The banks of the Shatt-al-Arab and of the lower Euphrates are continuously fringed with a belt of date-palms, 1 or 2 miles in depth, below which a carpet of coarse grass and other pasture plants is found and which cast a dense shade. From place to place marshland approaches the river banks and palm-trees give place to reeds and aquatic vegetation forming a canopy over sluggish creeks and muddy spits. The Tigris, on the other hand, is flanked from Qurnah northwards only by isolated groves of palms and other trees, as at Amarah, and its banks are for the most part lacking both in shade and in ground vegetation. During the spring flood all the country lying in the Basrah-Amarah-Nasriyah triangle merges with the Hammar and Senniyah lakes to become a single expanse of continuous marshland above which the banks of the rivers rise like islands. It so remains until late summer, and some parts of this vast region never dry out at all.

The air close to the river is thus very humid, especially near the ground, due to evaporation from the river itself and from the adjacent swamp.

Along the course of the Euphrates beyond Kurmah and of the Tigris beyond Amarah the palm belt gives way to scattered trees or ceases altogether, and except for short stretches the banks are no longer shaded; while owing to the absence of flanking marsh and the presence in its stead of arid desert the humidity is much lower. It is interesting to note that whereas in Basrah province there are over eleven million adult female date palms, in Baghdad province there are less than half a million (Sousa, 1944).

The occurrence of trichostrongylosis in man thus appears to be strictly confined to those portions of the banks of the main rivers where conditions of dense shade, high humidity and grass or other low ground vegetation are simultaneously found; since rainfall in the Tigris-Euphrates lowlands is slight (rarely exceeding 5 in. per annum) and confined to the winter and spring months of November to April.

Relative humidity figures were available only from Basrah and Baghdad but they are illuminating. In Basrah, where the incidence of trichostrongylosis in man is high, the mean monthly relative humidity never drops below 45%, and no doubt the microclimate close to the ground, aided by shade and the presence of grass and carpet plants, is much more humid. Crofton (1948) has shown that humidity is much higher near the base of grass-blades than near the tip and the rate of evaporation less. In Baghdad, on the other hand, where human trichostrongylosis is not found, during the six hottest months of the year the relative humidity is well below 40% and falls as low as 25% during the months of June, July and August (see Table 2). The rate of evaporation in Baghdad and other northern areas during these hot months must be very high, unchecked in most places by shade or ground vegetation. According to Crofton (1948) such conditions are lethal to the juveniles of *Trichostrongylus*.

Sheep and goats, the usual reservoir hosts of the species of *Trichostrongylus* commonly occurring in man, are abundant everywhere in the riverine areas of southern Iraq and southern Iran. Stewart (1949) records finding both ova and adult trichostrongyles in intestines of sheep obtained from the local slaughter-house on Abadan Island but did not identify the species. According to Marsh (1949), *T. colubriformis* was found in thirty-three persons in Abadan in the years 1933 and 1934; and is probably the species involved throughout the area. Further investigations on the occurrence of *Trichostrongylus* in sheep and goats and on the identity of the species in question are being carried out in Iraq, the results of which it is hoped to publish later.

The incidence of hookworm (*Ancylostoma duodenale*) infection, with which *Trichostrongylus* in man is so frequently associated, appears to rise as that of trichostrongylosis falls (see Fig. 2). In actual fact, however, this apparent rise is probably not significant since hookworm incidence, unlike that of trichostrongylosis, depends within certain broad environmental limits of temperature, humidity and soil type upon such exclusively human and social factors as extent and locality of soil pollution and the wearing of shoes, which may vary in different

parts of the same settlement. Unlike the incidence of trichostrongylosis, which is remarkably uniform, provide statistically significant figures. Suitable conditions for the development of hookworm juveniles,

Table 1. *Incidence of human trichostrongylosis and ancylostomiasis in southern Iraq*

Locality	Trichostrongylosis			Ancylostomiasis		
	Total examined	Number positive	Incidence (%)	Total examined	Number positive	Incidence (%)
Shatt-al-Arab:						
Basrah	335	85	25·4	335	37	11·1
Euphrates:						
Nasriyah	120	17	14·2	120	27	22·5
Kurmah	514	21	4·1	514	162	31·5
Felujah	95	0	0	95	6	6·3
Romadi	315	0	0	315	13	4·1
Hit	25	0	0	25	12	48·0
Canals taking off from the Euphrates above Hindiyah barrage:						
Hillah	50	0	0	50	6	12·0
Diwaniyah	75	1	1·3	75	43	57·2
Kerbela	116	3	2·6	116	34	29·3
Tigris:						
Amarah	250	9	3·6	250	49	19·6
Kut	236	0	0	236	31	13·1
Husainiyah	30	0	0	30	20	66·7
Zubaidiyah	50	0	0	50	24	48·0
Baghdad	1071	11	1·0	1071	107	10·0
Diyala:						
Baqubah	338	0	0	338	39	11·8

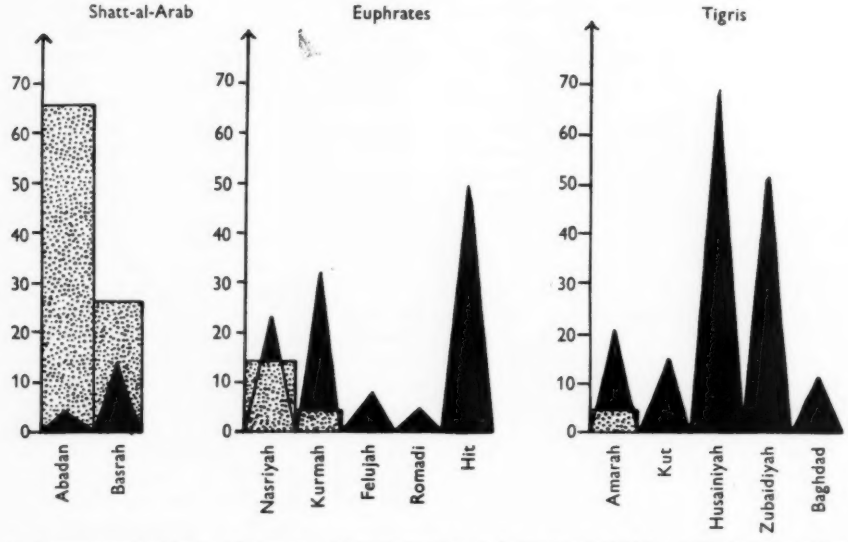

Fig. 2. Percentage incidence of human trichostrongylosis and ancylostomiasis in central and southern Iraq. Stippled rectangles, trichostrongylosis; black triangles, ancylostomiasis.

that of ancylostomiasis may vary widely within the same community and area; and larger samples than those obtained in this survey would be necessary to namely, patches of warm, shady, slightly damp, sandy soil contaminated with human faeces may be found here and there adjacent to dwellings and

Fig. 3. Map of the Tigris-Euphrates lowlands showing the localities mentioned and the extents of the marshy areas, ▭, and palm forest, ▭, in the south.

Table 2. *Mean monthly relative humidity* (1937–41 *average*) *after Sousa* (1944)

	Baghdad	Basrah
May	32	53
June	27	50
July	26	47
August	27	45
September	29	50
October	37	49

irrigation ditches in the vicinity of every settlement in southern Iraq. It is not surprising therefore to find that the incidence of *Ancylostoma duodenale* is everywhere considerable, despite the fact that the survey was largely confined to schoolchildren in the larger towns. A survey of village children in the same areas would probably have shown a higher incidence.

The fact that *A. duodenale* requires neither reservoir host nor intermediate host and that transmission is direct from man to man via such patches of suitable ground for larval development as may occur in the neighbourhood of favourite defaecation sites makes this infection largely independent of the climatic factors which limit the distribution of *Trichostrongylus* in this area.

Stewart (1949), in remarking that the conditions on Abadan Island are the reverse of those in which

ancylostomiasis would be frequent, has overlooked the fact that the occurrence of this infection depends upon the existence of such small, local patches of ground where conditions for juvenile development are satisfactory and human visitation is frequent. Many such patches were observed by the author on Abadan Island in the residential quarters of Ahmedabad and Bahmashir, south of the main refinery area, where the roads are not metalled and the majority of the population do not wear shoes. As Marsh (1949) has pointed out, damp soil suitable for the hatching and moults of *Ancylostoma* species is common in the riverine hinterland of Abadan, and the inhabitants habitually go about near their homes with bare feet. It may well be that the Anglo-Iranian Company's labourers were relatively free from this infection solely on account of the fact that they wore shoes. An examination of other lower income groups of the population of Abadan might have shown a higher incidence.

A factor which would tend to limit the occurrence of both ancylostomiasis and trichostrongylosis in some areas is the salinity of the soil where over-irrigation without drainage has taken place.

DISCUSSION

The restricted distribution of human trichostrongylosis in the Tigris-Euphrates lowlands is interesting when compared with the ubiquitous occurrence of ancylostomiasis. The explanation seems to lie in the different requirements of the free-living stages and their different powers of resistance to adverse environmental factors. Chandler (1925, 1926–7), and others, have claimed that hookworm juveniles have little resistance to desiccation which can be used as a method of controlling the infection. However, other workers have shown that *A. duodenale* is able to develop to the infective stage in the soil under relatively dry conditions and to resist considerable desiccation. Thus Clayton-Lane (1932) demonstrated, by means of earth cultures, that hookworm juveniles develop best when the water content is as low as 25 %, and that even when it falls as low as 9 % and the earth is powdery more than half the eggs may develop into vigorous infective forms. Similar results were obtained by Augustine (1923) in field experiments. This investigator found that after 10 days in drying soil in dense shade 52·4 % of infective juveniles still survived but the soil moisture had dropped to 10 %; under similar conditions but in light shade 34·6 % survived, although the soil moisture was only 3·5 %; whereas under similar conditions in direct sunlight all were dead within 5–10 days. He concluded that the minimum moisture which must remain in the soil to keep the infective juveniles alive was only 3 %. Heydon (1927) found that direct sunlight is fatal to exposed juveniles in 2 hr. De Nooij (1922) showed that hookworm juveniles could withstand desiccation in an incubator at 37° C. for 24 hr. and exposure to the sun for 1½ hr. It is clear, therefore, that drought will not lessen the infectivity of spots favoured for human defaecation provided that they are in partial shade.

Trichostrongylus juveniles, on the other hand, require high humidity and show a high death-rate when the rate of evaporation is great (Crofton, 1948). Moreover, since the infective forms climb the stems and leaves of grass and other carpet plants and this migration is favoured by low light intensity (Crofton, 1948), ground vegetation and shade, such as are found in the south of Iraq, are also necessary. Seghetti (1948) has shown that during the latter part of summer in Montana, when temperature is high and humidity low, 10 days exposure on the range is lethal to the majority of the infective stages; and Crofton (1948) has proved experimentally that in hot dry conditions, even at temperatures (69° C.) much lower than those obtaining in the valley of the twin rivers pre-infective forms of *Trichostrongylus* which hatch die within a week.

It is not surprising, therefore, to find that *Trichostrongylus* is confined to the marsh areas of southern Iraq where high humidity near the ground, a dense forest of palm trees shading the river banks and an abundant growth of grass, at any rate at certain seasons of the year, provide all the conditions necessary for the development of the free-living stages. Farther north, under drier and less-shaded conditions, this species is not found. *Ancylostoma duodenale*, on the other hand, being able to develop in the free-living stage in the absence of vegetation and in relatively dry conditions, is found not only in the south but also in the more arid central areas of Iraq along both banks of the Tigris and Euphrates.

The species of *Trichostrongylus* involved is not known since no adult specimens were recovered from human cases. *T. colubriformis*, *T. probulurus* and *T. vitrinus* are known to occur in Egypt (Looss, 1905) and probably extend their range to the Tigris-Euphrates basin also. *T. colubriformis* has been more frequently reported as a human parasite than the other species; and since it was found in human beings on Abadan Island by Marsh (1949), and in sheep in the same locality by Stewart (1949), it is almost certain that it is the species involved in the Iraqian cases also.

Reservoir hosts are probably sheep and goats which are common in the area and often live in intimate contact with man. Tsuchiya & Reller (1945) note that *Trichostrongylus* occurs as a human parasite principally in areas where ruminant hosts are plentiful; but Stoll (1947) has suggested that strains particularly adapted to the human host may have developed, although no experimental

proof of this hypothesis is yet available. Stewart (1949) believes that man-to-man passage of *Trichostrongylus* is established in southern Iran. This may well be the case in southern Iraq also, and investigations to check this point are being pursued.

Lie Kian Joe (1947) has shown that in Java the only portal of entry of *Trichostrongylus* into the human host is the mouth. Presumably, therefore, the infection is contracted in the Tigris-Euphrates area also by ingestion of greenstuff contaminated with the infective juveniles.

Since in Iraq and Iran, as in other localities where this infection occurs, human trichostrongylosis is often associated with ancylostomiasis care should be taken to differentiate the two parasites. *Trichostrongylus* ova are frequently mistaken for those of hookworms; and in view of the fact that the former is much more refractory to treatment than the latter such confusion may sometimes lead to prolonged and unsuccessful medication (Watson, 1946; Stewart, 1949). It is stated that in severe cases of trichostrongylosis in man emaciation, dry skin and mild anaemia may develop. It would be difficult to judge the pathogenesis and symptoms produced by this infection in the Tigris-Euphrates lowlands since multiple infestation with intestinal helminths is the rule, frequently complicated by urinary bilharziasis, malaria and malnutrition; but workers in other areas have generally considered it to be an essentially mild and symptomless infection (Lie Kian Joe, 1947; Stewart, 1949; Faust, 1949). Since light infections are usual and since no effective treatment is known for man, although tetrachlorethylene is stated to be very effective in sheep (Monnig, 1947), it would seem inadvisable to attempt medication except in proven severe cases.

In an earlier paper (Watson, 1946), it was remarked that isolated cases of human trichostrongylosis might well be pseudo-infections due to the ingestion of ova from the faeces of reservoir hosts on contaminated food or fingers. In the present instance, where a high incidence and wide distribution, explicable on environmental and epidemiological grounds, exist, there can be no doubt of the genuine nature of the infection. Lie Kian Joe (1947), in somewhat similar conditions in Indonesia, found an even higher incidence of this parasite in man and not only succeeded in recovering the adult worms at post-mortem examination but also was able to infect human volunteers experimentally.

Recent work has shown that species of *Trichostrongylus* are more common human parasites than had been originally supposed. Stoll (1947) estimated a world total of five and a half million infected persons of which three million were asigned to Japan and Korea, one and a half million to India, one million to Soviet Russia and insignificant numbers to the rest of the world. This figure is obviously an ultra-conservative estimate and needs revision. In Table 3 recent incidence data are correlated with local population figures. The resulting total of approximately fifty million infected persons, although probably nearer the truth than that of Stoll, is almost certainly still an underestimate. How easily the infection may be overlooked is illustrated by the fact that it escaped attention in the Malay Archipelago until 1939 despite surveys by competent investigators (Lie Kian Joe, 1947). As more data accumulate in the light of increasing awareness of the frequency with which this parasite occurs in man and is confused with hookworm the world total will probably rise.

Table 3. *Mean monthly maximum temperature* (1937–41 *average*) *after Sousa* (1944)

	Baghdad	Basrah
May	96	95
June	104	99
July	110	104
August	109	105
September	104	102
October	90	93

SUMMARY

An investigation of the occurrence of helminthic intestinal parasites in the population of central and southern Iraq led to the discovery that human trichostrongylosis is relatively common in man along the banks of the Shatt-al-Arab and the lower course of the Tigris and Euphrates rivers. In the Basrah area the incidence is 25·4 % and at Nasriyah 14·2 % but it falls rapidly along the northward course of the rivers and is absent in the centre and north of the country. This is correlated with the occurrence of heavy shade cast by palm forest, high humidity due to the presence of perennial swamps, and the occurrence of grass and other carpet plants in the south; which jointly provide the conditions necessary for the development and survival of the free-living stages of *Trichostrongylus*.

Hookworm (*Ancylostoma duodenale*) is more evenly distributed over the area and is common farther north where conditions are more arid; owing to the fact that the free-living stages can develop under much drier conditions than those of *Trichostrongylus*, probably mainly on moist defaecation sites near irrigation channels.

The species of *Trichostrongylus* involved is not known but is probably *T. colubriformis* which is common locally in sheep and has been found in man at Abadan by Marsh (1949). Infection is presumably contracted by ingestion of greenstuff carrying infective juveniles.

Since infections are light, symptoms minimal and treatment ineffective care should be taken to

differentiate trichostrongylosis from ancylostomiasis in order that no unnecessary medication be undertaken.

The world incidence of human trichostrongylosis is higher than was formerly believed. The available reports are reviewed, and it is concluded that not less than fifty million persons harbour this worm throughout the globe.

Table 4. *Estimated world incidence of human trichostrongylosis*

Area	Population	Incidence (%)	Number infected	Authority	Date
Far East:					
Korea	25,000,000	23·6 (1)	5,900,000	Hunter, Ritchie *et al.*	1948–51
Japan	84,000,000	10·3 (2)	8,652,000	Hunter, Ritchie *et al.*	1948–51
China	466,582,000	1·0 (9)	4,665,820	Estimate	—
Indonesia	75,000,000	41·2 (4)	30,900,000	Lie Kian Joe	1947
India	389,000,000	1·0 (5)	3,890,000	Chandler	1926, 1927
				Sweet *et al.*	1929
				Maplestone	1941
Mauritius	500,000	8·0 (3)	40,000	Webb	1937
Middle East:					
Southern Iran	500,000	64·8 (6)	324,000	Stewart	1949
Southern Iraq	1,750,000	11·8 (7)	206,500	Watson	1952
Africa:					
Belgian Congo	11,000,000	10·0	1,000,000	Van Slype	1934
Rest of Africa	158,000,000	1·0 (8)	1,580,000	Estimate	—
U.S.S.R.:					
All areas pooled	—	—	1,000,000	Stoll	1947
		Total	58,158,320		

Notes:

(1) Hunter and his colleagues based their estimate of incidence on investigations in South Korea, but it is not unreasonable to assume that they apply to the whole peninsula.

(2) This incidence figure is an average obtained from ten separate investigations in different prefectures in which the results ranged from 0·5 to 44·4%. In some villages the incidence was as high as 82·6%.

(3) The survey quoted was carried out on the Island of Mauritius but the population figure applies to the whole group. The infection is presumably not confined to the one island.

(4) Lie Kian Joe's investigations were carried out by post-mortem examination in Batavia, but the results may not unreasonably be extended to cover the whole archipelago; since climatic and other conditions throughout the area would appear to be suitable for the free-living stages of *Trichostrongylus*.

(5) The various investigators quoted found incidence rates ranging from less than 1% to over 25% in different districts, but the consensus of their opinion was that not less than 1% of the population of the sub-continent could be considered to be infected with this parasite.

(6) The figures given by Stewart applied to labourers working and living on Abadan Island which has a population of about 250,000; but if we take into account the similar conditions obtaining in Khorramshah, Ahwaz, and other neighbouring districts on the east bank of the Shatt-al-Arab we arrive at the higher population figure.

(7) The incidence figure here is the mean of those found at Basrah, Nasriyah, Amarah and Kurmah, and the population figure that of the provinces of Basrah, Amarah, Muntafik, Diwaniyah, Kerbelah and south-eastern Dulaim which flank the rivers in the infected zones.

(8) In view of the high incidence found in the Belgian Congo by Van Slype it seems illogical to suppose that human trichostrongylosis does not occur fairly extensively in other parts of Africa.

(9) In view of the high incidence found in Korea and Japan by Hunter *et al.* it seems probable that the infection is also widespread on the Chinese mainland; indeed, 1% is more likely to be an underestimate than an overestimate.

(10) Population figures were taken from the 1952 edition of Whittaker's *Almanac*, with the exception of those for Korea and Iraq which were supplied by Hunter *et al.* and the Iraqi Government Census Department respectively.

(11) It is probable that human trichostrongylosis occurs also in other areas of the world not listed above, but since only isolated case records are available it is impossible to make any estimate of its frequency therein.

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THE LABORATORY MAINTENANCE OF *GLOSSINA*. I

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(With 7 Figures in the Text)

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I. INTRODUCTION

The experiments on methods of maintenance of *Glossina* which this paper describes were carried out with the special objective of devising means whereby large numbers of tsetse-flies could be maintained as stock populations for other experimental purposes, especially research in trypanosomiasis. Maintenance by methods which could not be applied to large numbers, such as keeping flies singly in tubes, was not undertaken.

Work previously reported on maintenance of tsetse-flies (Roubaud, 1917; Broom, 1939; Geigy, 1948) either has been on a small scale or has included no indication, such as may be obtained from records of longevity or pupal weight, of the general condition of the flies during the maintenance. In the present experiments an attempt was made to collect sufficient data to provide reliable assessments both of the results of any modifications of technique and of the general well-being of the populations at any given time.

Tsetse-flies are kept in a number of laboratories in tropical Africa but, in general, the flies are of a locally available species, and numbers are kept up by regular reinforcements of wild pupae or flies or of pupae from captured wild flies. The present experiments were begun in the hope that it might eventually prove possible to maintain flourishing cultures of several different species of *Glossina* obtained initially from widely separated places, thus avoiding the difficulties of obtaining pupae from remote districts by post and the losses—often serious—inherent in this method of supply.

As different species of tsetse require very different climatic conditions, it seemed that the best approach would be to attempt to maintain the locally occurring species first, to pass from this to closely related species and to graduate from these to species from areas with very different climates. Following this plan, by far the greater proportion of the work here reported was done on *G. swynnertoni* Austen, but experiments on a smaller scale have also been made with *G. morsitans* Westwood, *G. pallidipes* Austen, and *G. austeni* Newstead.

The experiments fall into three general groups, each following from the last but with some overlap in time. In the first group a general study was made of death-rates and reproduction-rates under the most simple conditions of maintenance in the laboratory, the population being constantly reinforced or increased by flies brought in from the bush. The second group consisted of a series of experiments—several of them cut short by accidents—in which

simultaneous comparison of various factors in maintenance was made and the effects studied on such points as longevity of the female flies, numbers of pupae obtained, mean weights of pupae and derived data such as the number of pupae per 100 female-fly-days. These first two sets of experiments were all on *G. swynnertoni* and from them developed the third group in which the lessons that had been learnt were applied to an 'all-out' attempt to maintain a flourishing population of *G. swynnertoni*, and small pilot experiments were made with populations of *G. morsitans*, *G. pallidipes* and *G. austeni*. In addition, during the course of these experiments, a quantity of data was obtained on weight-loss of pupae from pupation to emergence, and a section is included describing the more important results from pupae of *G. swynnertoni*, to which the great majority of these data referred.

Shortly after beginning this work the writer had the great advantage of meeting Prof. R. Geigy of Basle, who was then on a visit to East Africa, and of hearing from him of his difficulties and successes in the maintenance of a population of *G. palpalis* Robineau-Desvoidy. Later, when he had returned to Switzerland, Prof. Geigy was generous enough to send a complete feeding-rack of the type designed and used by him for feeding several cages of flies simultaneously on guinea-pigs, and this rack has been used throughout for all feeding on these animals. A full description is to be found in his paper (Geigy, 1948). The writer wishes to express at the outset his gratitude to Prof. Geigy for his helpful advice and for his generosity.

II. THE FIRST MAINTENANCE EXPERIMENT

For the first experiment on the laboratory maintenance of *G. swynnertoni* non-teneral female flies were brought in from the bush and kept in 'Geigy' cages made by fitting a mosquito-gauze sleeve over a wire frame of approximately 15 × 8 × 5 cm. outside dimensions. The number of flies kept in one cage varied from five to fifteen and the cages were kept standing on end in high-sided boxes over water. Each box was kept on a small cardboard tray or Petri dish with one edge lifted on the side of the tray or dish so that there was room underneath for a larva, when deposited, to pass through the meshes of the mosquito-gauze and pupate in the tray. By this means pupae could be collected without the disturbance entailed in extracting them from the cage. The flies were offered food daily by applying the cages to the shorn flank of a sheep which was held lying down, in a small crush. All cages were kept in position for at least 30 min.

At first the flies were kept in the laboratory during the months of November and December when the minimum (night) temperature rarely falls below 22° C. and usually varies between 23 and 25° C. depending on the incidence of rain, and the mean maximum falls from over 32° C. in October to about 26° C. in December again with the advent of rain. During the hot dry weather immediately preceding the rains the death-rate ranged from 10 to 20 % daily but, with close supervision of feeding methods and the cooling effect of the early rains, it ranged from 5 to 10 % and later below 5 % per day. The death-rate was never low enough to make the maintenance of a population at all practicable.

Some cages were then kept outside the laboratory standing in high-sided boxes over water in a grass-roofed, open-sided hut. They were shaded from any direct sunlight and insulated from ants and other predators by water barriers. An immediate improvement in longevity resulted; the mean length of life of a batch of flies kept this way was 34·55 days from being brought in from the bush compared with 22·21 days for a control group kept in the laboratory.

At the same time some small experiments were done on the effect of crowding, and, though the death-rates in cages containing eleven to fifteen flies were greater in proportion than in the less crowded cages, the difference was not statistically significant. At ten or less per cage no effect could be detected and it was concluded that, in a cage of the dimensions given, ten flies could be kept without suffering from overcrowding. In passing, it may be remarked that, for an insect as active as the tsetse-fly, the consequences of mere confinement in a small space might be expected to be far more serious than any further effect of crowding, but must unfortunately be accepted as part of any maintenance on these lines.

III. THE ORTHOGONAL MAINTENANCE EXPERIMENTS

(*a*) *Design and conditions*

Several experiments, all planned on a full orthogonal design, were made to compare three conditions of maintenance and two methods of feeding simultaneously, with each feeding-maintenance combination replicated. The conditions of maintenance were: at constant 26° C.; in the laboratory (for comparison with previous results), and in the outside hut described above. The methods of feeding were: (1) on guinea-pigs in the 'Geigy' feeder, and (2) on sheep in the crush already mentioned except that, in the meantime, it had been found better to place the cages of flies side-by-side round the flanks and abdomen of the sheep and strap them in this position with a long rubber band. By this means the cages were held firmly but with sufficient flexibility to prevent them sliding over the skin with slight

movements of the animal, so obviating the risk of breaking off the proboscis of a fly feeding through the gauze covering.

It had originally been intended also to include tests of the effect of keeping flies inactive at 8° C. for 48 hr. before beginning maintenance. Prof. Geigy had adopted this means of keeping flies quiescent when taking them home by air from West Africa to Switzerland, and it might conceivably have had an effect in acclimatizing flies to capture and confinement. However, the mortality in flies exposed to this treatment proved to be so great that it was impossible to distribute daily catches of flies from the bush equally between the twelve cages needed for all the permutations of maintenance and feeding in a 'cooled' and 'not cooled' series. It was therefore decided to use the extra six cages as replications of the maintenance-feeding permutations.

The twelve cages of each experiment can therefore be represented diagrammatically thus:

	All fed on guinea-pig		All fed on sheep	
	1st replication	2nd replication	1st replication	2nd replication
Maintained at constant 26° C.	CI*a*	CI*b*	CII*a*	CII*b*
Maintained in laboratory	LI*a*	LI*b*	LII*a*	LII*b*
Maintained in outside hut	OI*a*	OI*b*	OII*a*	OII*b*

It was foreseen that flies brought in on different days might be subjected to very different climatic conditions during the period of catching and transport. In order to avoid false results in comparisons of longevity due to this cause, only multiples of twelve flies were distributed amongst the twelve cages each day until the full 120 had been obtained. Any residue over a multiple of twelve on any day was discarded. Thus, in the first of these experiments, flies were obtained and caged as follows: 1st day—over twelve, one in each cage; 2nd day—over thirty-six, three per cage; 3rd day—less than twelve, none added; 4th, 5th and 6th days—over twenty-four each day, two per cage each day. In this and later experiments no fly died during the period of building up the numbers in the cage.

The data recorded from the experiment were:

(1) The number of flies alive in each cage daily until the death of the last fly.

(2) The date of deposition and emergence of each pupa.

(3) The weight of each pupa when first obtained and daily for ten weighings.

(4) Pupa weights on alternate days after the first ten weighings until emergence.

From these data a number of derived quantities could be obtained, such as:

(5) Mean initial weights of pupae from each cage.

(6) Number of pupae per 100 fly-days from each cage.

(7) Weight of pupae per 100 fly-days from each cage.

The data on weight-loss of pupae will be considered later in the section on the maintenance of pupae.

Several experiments were started and each was given a code letter for distinct marking of cages of flies. These were:

A. An experiment with the above orthogonal design of twelve cages of ten *G. swynnertoni* each which went on to the death of the last fly.

B. A replication of 'A' started 7 days later but interrupted after 32 days by ants getting into one cage and killing all the flies.

C. An experiment to test the effect of the 48 hr. cooling at 8° C. mentioned above. Survivors were compared with 'uncooled' flies in survival and rate of reproduction but no difference approaching a significance level of $P = 0{\cdot}05$ was found in any of the data.

D. Another attempt to replicate 'A'. Again cut short by ants but at only 17 days.

E. A third attempt to replicate 'A', suffering the same fate at 22 days.

The losses by ants occurred in each case from a single straw or piece of grass being blown from the thatch of the hut across the edges of the water barrier. Vigorous steps were taken both to attack the ants and to prevent such short-circuits recurring, and thereafter losses have occurred on rare occasions but have not seriously interfered with maintenance. Although the survival records of three experiments had been cut short, a large number of pupae had been obtained from them and the combined data provided much useful information which was consistent with that from Exp. 'A'.

(*b*) *Results*

The findings from these experiments relating to the original female flies brought in from the bush are shown in Table 1.

The very poor results of maintenance at a constant temperature of 26° C. must be attributed chiefly to the dryness of the atmosphere in the constant temperature cabinet used. As this temperature lay within the range of daily variation the only means, with the apparatus available, of maintaining it constantly was to cool the air below the minimum daily temperature and vary the amount of

Table 1

Conditions of maintenance or feeding	Survival (days)* Mean	Survival (days)* Max.	Mean weight of pupae† (mg.)	Pupae per 100 fly-days†
At constant 26° C.	26·2	58	22·33	3·51
Inside laboratory	34·8	68	23·91	4·73
In outside hut	43·4	92	24·41	3·47
Sheep fed (all maintenances)	33·6	—	23·95	3·95
Guinea-pig fed (all maintenances)	35·4	—	23·46	3·85

* From Exp. 'A' only.
† From Exps. 'A', 'B', 'D' and 'E' combined.

reheating to keep the temperature at the required level. The resultant removal of moisture by the cooling unit was too great to obtain an adequate humidity, even in an almost completely closed box over water. It should be noted also that 26° C. is above the mean temperature of the habitat of *G. swynnertoni*; this is in the range 23–24° C.

One result from an analysis of variance of the combined data from all the survival results should be recorded and that was a significant first-order interaction between methods of maintenance and methods of feeding. Flies maintained in the laboratory did slightly better when fed on guinea-pigs and those maintained outside were better when fed on sheep. This point will be referred to again at the end of the discussion on the long-term maintenance experiment on *G. swynnertoni*.

The information obtained from the data on later laboratory-bred generations in these experiments is of little interest as it is better and more fully available from the later experiment in which the experience gained so far was utilized in an attempt to maintain a flourishing population of *G. swynnertoni*.

IV. EXPERIMENTS ON LONG-TERM MAINTENANCE

The third stage in the series of experiments on maintenance of *Glossina* was to apply the lessons already learnt to an attempt to maintain a population of *G. swynnertoni* over a long period and also to determine what conditions were suitable for other species. In all these later experiments detailed data were collected and each generation was kept separate, as one of the main requirements was to obtain direct comparisons in such important criteria as longevity, weights of pupae and others discussed below. Because the comparisons of guinea-pig feeding and sheep feeding had given inconclusive results in the earlier experiments it was decided to continue this investigation by dividing the population equally between the 'host' animals.

V. MAINTENANCE OF *GLOSSINA SWYNNERTONI*

An experiment with *G. swynnertoni* was begun early in February 1951, with the special intention of completing at least a full year of maintenance under laboratory conditions, as it would be of value to determine to what extent recovery could take place during the rains from the severe effects to be expected in the later and hottest part of the dry season.

For easy reference, Fig. 1 shows, at the top, the chief meteorological data throughout the period of these experiments and, below, the succession in time of the generations in the experiment on *G. swynnertoni* and also in the later experiments on *G. morsitans*, *G. pallidipes* and *G. austeni*. The hachured bars show the period of life of each generation of flies from the emergence of the first to the death of the last, and the inset solid bars represent the periods of pupal life from the first deposition to the last emergence.

(a) *Conditions of the experiment*

Sixty non-teneral female *G. swynnertoni* were brought in from the bush and kept in the grass-roofed hut outside the laboratory in 'Geigy' cages (Geigy, 1948, fig. 2) with ten flies in each cage. The cages stood on end over water in high-sided boxes, each in its own small cardboard tray and slightly tilted to leave space for deposited larvae to pass through the gauze of the bottom of the cage and pupate in the tray where they were readily available for collection. The flies were offered food daily, thirty on guinea-pigs in the 'Geigy' feeding-rack in the laboratory and thirty on sheep in the outside crush.

In later generations the sexes were segregated with a maximum of ten flies per cage. Females which emerged from pupae in the experiment were paired with a male of at least 9 days of age immediately after their first feed. Pupae were collected daily from each set of cages of females and kept singly in labelled $3 \times \frac{1}{2}$ in. tubes in the laboratory at a constant relative humidity of 90%. As will be seen in the section on maintenance of pupae this

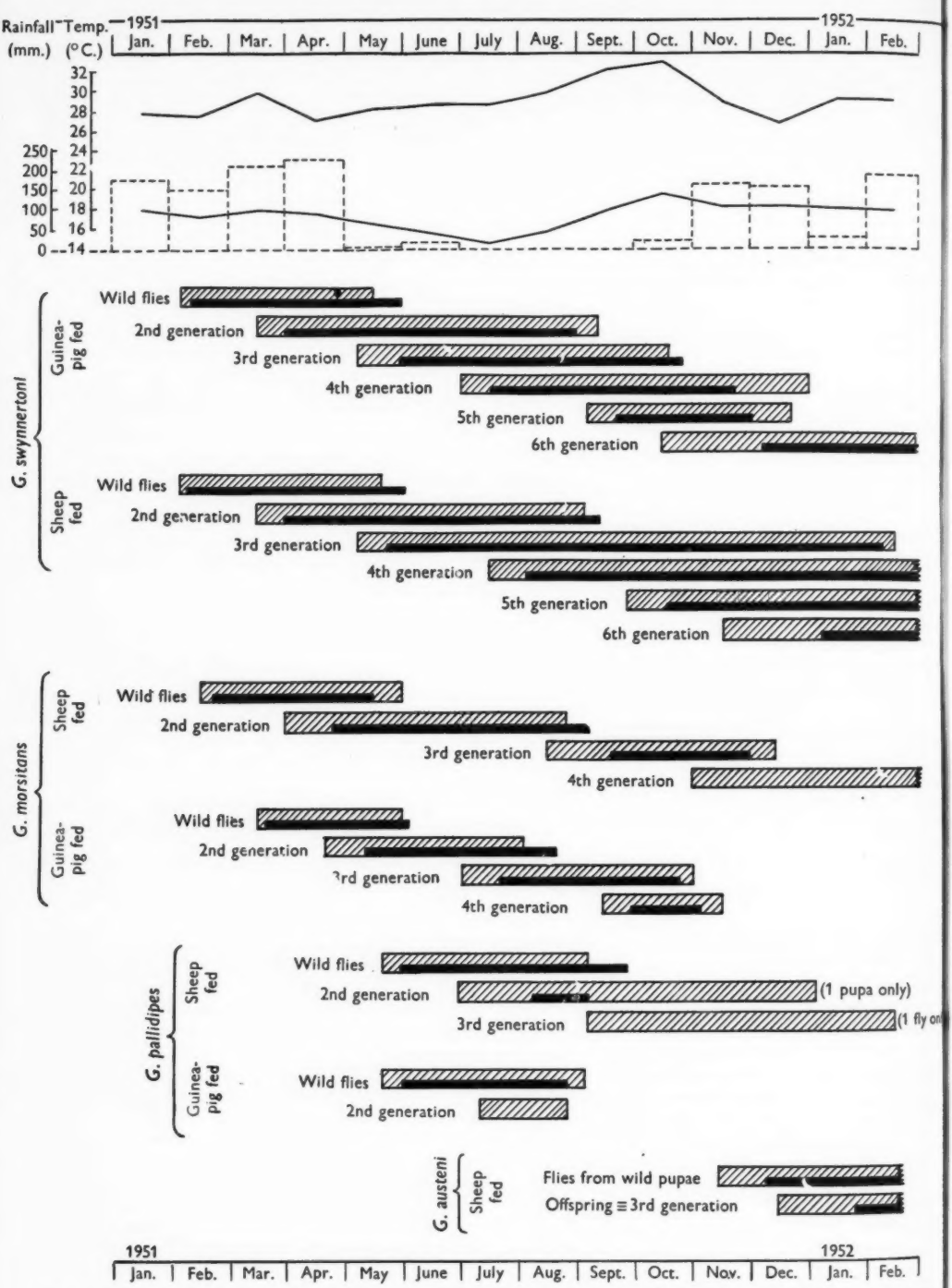

Fig. 1. The uppermost part of the figure shows the climatic conditions during the period of the long-term maintenance experiments. Monthly rainfall and mean maximum and minimum temperatures are shown, the horizontal scale being divided equally by months. The lower part shows the time-relationship of the various experiments described later and the succession of the generations within each experiment. Lives of females only in each generation are shown, the hachured bars representing the periods from the emergence of the first female in each generation to the death of the last, and the inset solid bars representing the periods of life of the pupae from those females from the first larvi-position to the last emergence.

type of maintenance greatly decreased weight-loss while gaining the advantage of the shorter pupal period in the higher laboratory temperatures.

(b) *Data recorded*

The following data were collected:

Longevity. The number of flies alive in each cage was noted each day.

Weights of pupae. All pupae were weighed within 24 hr. of pupation. In the early part of the experiment this first weighing was followed by daily weighings up to a total of ten and thereafter by weighings on alternate days until emergence. Later this system was changed to weighings on the first and sixth days (covering the early period of rapid weight-loss) and daily at the end of the pupal period in order to obtain more precise information on final weights before emergence.

Fertility of females. All females were dissected when found dead and the spermathecae examined for insemination.

(c) *Results and discussion*

The main results of this experiment are set out in Tables 2–4. Table 2 gives the data on fly-lives, with means and longest lives.

(1) *Longevity*

The most striking feature of these data in Table 2 is the comparison between the mean lengths of life in guinea-pig-fed and sheep-fed flies. In neither of the two cases where the mean life in guinea-pig-fed flies is slightly the longer (the original wild flies and the second generation males) is the difference statistically significant, but in all the remaining cases, where the 'sheep-fed' life is the longer, the difference is significant with a very low level of probability. Further, not only does the mean life of the guinea-pig-fed flies never reach the high levels achieved later in the sheep-fed flies, but also, after reaching a moderate value in the second and third generations, it declines steadily, whereas the 'sheep-fed' mean lives remain long.

In one cage of ten female sheep-fed flies of the fourth generation an average life of 110 days was achieved, and this over the height of the dry season from mid-September onwards. At the time of writing there is no indication of any falling-off in longevity in the sixth and later generations, but the data from these are still far from complete.

The comparison between male and female lives is also interesting in that the excess of female mean life over male mean life is remarkably constant. Only once (guinea-pig-fed second generation) does the male mean life exceed the female mean life and there the difference is quite insignificant. There is also one example of an insignificant excess of female mean life over male mean life (sheep-fed third generation). The extra length of life of the females in the remaining six pairs of data lies always in the range 20–26 %.

Table 2. *Longevity data of laboratory-maintained* Glossina swynnertoni

		Guinea-pig-fed				Sheep-fed			
	Sex	No. of flies (1)	Total fly-days (2)	Mean life (days) (3)	Longest life (days) (4)	No. of flies (5)	Total fly-days (6)	Mean life (days) (7)	Longest life (days) (8)
Original wild flies	♀♀	30	1503	50·1	97	30	1403	46·7	102
2nd generation	♂♂	25 (14)	924	66·0	120–137	21 (13)	848	65·3	103–138
	♀♀	40	2613	65·8	106–112	30	2479	82·6	102 122
3rd generation	♂♂	53	2994	56·5	108–131	29	2454	84·6	149–191
	♀♀	32 (23)	1616	70·3	89–114	33 (29)	2557	88·2	157–174
4th generation	♂♂	21	941	44·8	63–113	64	4579+	72+[75]	—
	♀♀	40	2237	55·9	104–132	52	4211+	81+[90]	—
5th generation	♂♂	26	942+	36+	—		Data incomplete		
	♀♀	19	828	43·6	76–98		Data incomplete		

Notes. (1) A second entry in brackets under 'no. of flies' indicates the number left after some flies had been taken for fat estimation or lost by escape or accident. The subsequent entries in cols. 2–4 or 6–8 are based on the data for the flies remaining.

(2) The ranges given for maximum life allow for the period in which emerging flies built up the number in the cage. As flies were not individually distinguishable the last to die may have been any from the first to the last to emerge.

(3) The data followed by a '+' are as yet incomplete. A further entry in square brackets represents an estimate of the final value.

(2) *Reproduction*

In Table 3 are given the data on reproduction in each generation in terms of the total number of pupae obtained, the mean interlarval period and the number of pupae per female fly.

It is at once obvious from these data that the comparison of guinea-pig-fed and sheep-fed flies shows the same trend as has been remarked in the longevity data. At first the guinea-pig-fed flies give better figures but later they fall away badly, whereas the sheep-fed flies show a steady improvement throughout. This trend is especially apparent in the number of pupae per fly. Clearly, if the population numbers are to be maintained, this number must exceed two for, in addition to the need to reproduce the full number of males and females, there must be an excess to allow for the wastage of a certain proportion of markedly underweight pupae which always occur. Further reference to this point will be made in the discussion of the data in Table 4.

Table 3. *Reproduction data of laboratory-maintained* Glossina swynnertoni

	Guinea-pig-fed				Sheep-fed			
	No. of females (1)	Total no. of pupae (2)	Mean interlarval period (3)	No. of pupae per female (4)	No. of females (5)	Total no. of pupae (6)	Mean interlarval period (7)	No. of pupae per female (8)
Original flies	30	83	15·64	2·77	30	63	19·02	2·10
2nd generation	40	113	20·76	2·83	30	96	24·21	3·20
3rd generation	32	89	20·56	2·78	33	147	18·87	4·45
4th generation	40	76	25·36	1·90	52	207+	20·37	3·98+
5th generation	19	39	20·87	2·05	Data incomplete			

(3) *Interlarval period*

The second outstanding point in these data is the great length of the mean interlarval periods, which are far longer than the known interlarval periods for tsetse flies at these temperatures (Mellanby, 1937; Jack, 1939), and are mostly of the order of twice the expected length. Thus Mellanby (1937) found 10 days for *G. palpalis* at 24° C. and Jack (1939) 11 days for *G. morsitans* at the same temperature. The figures in Table 3 were not calculated by simple division of the total number of fly-days by the total number of pupae as this would have taken no account of the barren period at the beginning of each female's life. In order to make proper allowance for this period of infertility the linear regression of 'number of pupae' (Y, dependent variate) on 'number of fly-days (X, independent variate) was calculated in the form

$$Y = \overline{Y} + b\,(X - \overline{X}),$$

where $\overline{Y}$ and $\overline{X}$ are the mean values of Y and X. By this method the best estimate was obtained of both the mean interlarval period (which is the reciprocal of 'b') and the initial barren period of which the value of X when $Y = 0$ gives a measure. The interlarval periods quoted in cols. 3 and 7 of Table 2 are the reciprocals of 'b' from each calculated regression.

(4) *Insemination of laboratory-maintained females*

It had already been noted in earlier experiments that the number of pupae was small for the number of female fly-days and, in order to obtain more information as to the cause, all females in this experiment were dissected as soon as they were found dead and the spermathecae examined for insemination. Up to the present, 293 *G. swynnertoni* have been examined and of these 35 were too dry, or otherwise unsuitable, for a definite opinion to be given. Of the remaining 258, 236 proved to be fully inseminated, twenty were partially inseminated and only two were uninseminated. It is, therefore, clear that failure of insemination was not responsible for the proportionately small number of pupae, and it must be presumed that, in their condition of captivity, the females aborted very frequently, the proportion of abortions being usually of the order of 50%.

Although, from the conditions of the experiment, the identification of pupae from individual flies was usually impossible, it was occasionally possible at the tail-end of a generation. A number of the identifiable pupae periods lay in the expected range of 10–13 days, but some were of nearly twice this length, suggestive of an intermediate abortion. It could not be decided from the data whether the reduced number of pupae was due in the main to a fairly constant abortion-rate in the majority of the flies or to a high abortion-rate in a minority.

(5) *Initial weight of pupae*

It was mentioned above, in connexion with the number of pupae per female fly (Table 3), that some very small pupae were always obtained. The

weights of these appeared, even by inspection, to be outside the range of a normal distribution, and their occurrence is shown in the histograms in Fig. 2 in which there is a general tendency to a longer tail on the side of the lighter pupae. The symmetry of the weight-distribution of the pupae from the original wild flies was tested by calculating 'g_1' from the third cumulant of the distribution. The result, $g_1 = -0{\cdot}502 \pm 0{\cdot}201$, showed significant negative skewness ($P < 0{\cdot}02 > 0{\cdot}01$).

unduly low as all pupae are included with the exception of an occasional pupa of very low weight which died within a few days of pupation, and, as only flies from the pupae of 22 mg. or over were kept, the second bracketed entries under 'mean weight' give true measures of the relative sizes of the flies comprising the next generations.

Turning to the general trend in the initial mean weight and the comparison between data from guinea-pig-fed and sheep-fed flies we see once again

Table 4. *Data relating to pupae from laboratory-maintained* Glossina swynnertoni

	Guinea-pig-fed					Sheep-fed				
	Mean weight (mg.) (1)	Total no. (2)	No. emerged (3)	No. over 22 mg. (4)	No. emerged over 22 mg. (5)	Mean weight (mg.) (6)	Total no. (7)	No. emerged (8)	No. over 22 mg. (9)	No. emerged over 22 mg. (10)
From original flies	26·71 (27·79)	83	80	72	70	25·81 (27·04)	63	61	53	53
From 2nd generation	26·95 (28·27)	113	106	96	92	24·87 (26·71)	96	87	72	66
From 3rd generation	25·48 (27·09)	89	76	72	66	25·07 (26·28)	147	142	124	122
From 4th generation	23·33 (24·78)	76	62	60	53	23·98 (25·46)	198+	184+	148+	142+
From 5th generation	23·67 (24·92)	39	35	31	29	[24·34] (26·07)	[99+]	[90+]	[73+]	[69+]

Notes. (1) The bracketed second entries in cols. 1 and 6 are the mean weights of all pupae over 22 mg. (see text).
(2) The figures in square brackets for pupae from the fifth sheep-fed generation refer to the first ninety-nine pupae only, for which full data are at present available. At the time of writing this generation gives every indication of continuing the increase shown in previous generations (see text and Fig. 2).
(3) The data for the pupae from the fourth sheep-fed generation are taken from the first 198 which have, so far, emerged or died.

Also, in several of the histograms in Fig. 2 there is a deficiency of pupae in the range 20·0–21·9 mg. and a larger number just below this range. This fact, backed by the skewness of the weight-distribution, was taken to indicate that pupae of initial weight of less than 22 mg. were not part of a population but were 'runts'. As these might be expected to be much more liable to elimination in nature, it was decided to keep only flies emerging from pupae of initial weight of 22 mg. or over in the laboratory population. The weight distribution of pupae above this 'bar' was tested and found not to depart significantly from symmetry (by 'g_1') or normality (by 'g_2').

The entries in Table 4 showing 'number of pupae above 22 mg.' and the number of emergences from them, and the second entries under 'mean weight' (cols. 1 and 6) follow from the result of this investigation of the normality of distribution of initial weights of pupae. The first entries under 'mean weight' are

the same result as has been pointed out in the data in Tables 2 and 3 though the effect here appears later than before. The pupae from the guinea-pig-fed flies are of greater mean weight than those from the sheep-fed flies for the first three generations, the greatest difference being in the second generation. By the fourth generation the sheep-fed flies are giving the better results and, from the aspect of maintenance of a population, doubly so, for they are steadily increasing their numbers, whereas the decline in the guinea-pig-fed population has set in. The fall in mean pupal weight which occurred in both populations at this time may be attributed, at least in part, to the severe climatic conditions of the hot dry season during which most of these pupae were deposited. The mean weights increased in the next generation in both feedings, and the greater increase occurred in the sheep-fed fifth generation which came later in time (see Fig. 1)—almost wholly after the break of the rains. Assessing these results

in terms of the main objective of these experiments—the maintenance of tsetse populations for experimental work in trypanosomiasis—the sheep-fed population is seen to be at least keeping up a full complement of flies of the same weight as the original wild flies or actually increasing their number. At the same time many extra flies of a rather smaller size are being produced, and it should not be difficult to establish in experiments on trypanosomiasis whether results from these flies were comparable with those either from flies from wild pupae or from larger laboratory-bred flies.

Maintenance over a full year has, in the sheep-fed population, shown the beginnings of the hoped-for recovery with the coming of the rains, though it is too early to say whether the rise in mean pupal weight will continue until the early data are equalled. The decline in all the data from the later generations of the guinea-pig-fed flies is so marked that this population seems doomed to extinction.

(6) *Emergences from pupae*

The difference between the two populations is again brought out very clearly by the figures in Table 4 for emergences from pupae of over 22 mg., which were the numbers of flies (with the exception of occasional early deaths) which went to form the next generation. With the hot season fall in mean pupal weight the proportions of pupae of weight above 22 mg. decreased, and in the guinea-pig-fed population this only exaggerated the decline in numbers. In the sheep-fed population, on the other hand, the reproduction-rate was so good that, despite this extra drain on the population, the numbers continued to increase. The smallest proportional increase coincided with the decrease in pupal weight attributed above to the most severe climatic conditions of the year.

Information is also available from Table 4 on the proportions of emergences from pupae of both populations and also from those above and below 22 mg. initial weight, as the data are given in such a form that the total numbers of emergences and failures can be extracted for all the pupae, for the pupae over 22 mg. and for those under 22 mg. The results of comparisons by 2×2 χ^2 tables between the figures for pupae from the guinea-pig-fed and sheep-fed flies were: for all pupae $\chi^2 = 4{\cdot}69$, $P < 0{\cdot}05 > 0{\cdot}02$; for those above 22 mg. $\chi^2 = 2{\cdot}65$, $P < 0{\cdot}2 > 0{\cdot}1$, and for those below 22 mg. $\chi^2 = 4{\cdot}89$, $P < 0{\cdot}05 > 0{\cdot}02$. In each instance the guinea-pig feeding gave the lower proportion of emergences. All the comparisons between pupae above and below 22 mg.—for all the pupae and for guinea-pig-fed and sheep-fed separately—showed a markedly lower emergence rate from the smaller pupae, with high values of χ^2 and probabilities far below 0·001.

(d) *General discussion*

The distributions of initial weights of pupae from the various generations are shown in the histograms in Fig. 2 in which the trends in mean weight and numbers can be seen.

The guinea-pig-fed population started at a rather higher level than the sheep-fed but later suffered a marked shift to the left (decrease of pupal weight) with reduction of numbers, whereas the sheep-fed population, though a little lower at first, showed but little decline in size and a steady increase in numbers. In order to avoid a false impression of a large fall in numbers in the sheep-fed fifth generation the tops of the histograms have been left dotted as incomplete and a dotted curve has been put in to represent the minimum expectation from this generation. Previous experience had shown that the maximum rate of production of pupae occurs, as would be expected, when about half the final number of pupae has been obtained. During the period of writing up the results for this paper this fifth generation has been producing pupae at such a rate that, in the time between bringing the results up to date for Table 4 and Fig. 2 and the actual writing of the discussion of those results, the number of pupae had risen from 99 to 153 with a high proportion of large pupae in the last 54. Further, as the rate of production of pupae still showed no sign of decreasing, it is reasonable to presume that the total number will finally exceed 300, and the dotted curve in Fig. 2 is based on this number as an estimate.

The rise and fall of the female populations, guinea-pig-fed and sheep-fed, for each generation, is shown in Fig. 3. In Fig. 1 the horizontal scale was divided into equal parts by months, but in Fig. 3 the slight inaccuracy introduced by this type of scale is avoided and the horizontal divisions represent strict periods of 10 days each. In addition to the number of female-fly-days recorded in each period the number of pupae deposited is shown as a small inset column. Since the periods are all of 10 days, the mean number of flies alive in any period is easily seen by taking the vertical scale as ten times larger, and then the mean numbers of flies alive and the number of pupae obtained are both on the same scale.

Several points worthy of comment are brought out by this figure. At the beginning of each of the histograms for captured wild flies there is a conspicuous lag of 'number of pupae' behind 'number of flies alive', and the proportion of these two to each other does not reach its usual order until the third 10-day period when the number of pupae per 10-day period also reaches a peak. Had there been any reason to suppose that many of the flies were very young when caught, this finding would have been

expected, but the fairly even spread of deaths over the next 100 days suggests that the flies were randomly distributed as to age at the time of capture. The conclusion is, therefore, that the actual fact of capture and confinement either upset the normal rhythm of gestation or caused a high proportion of

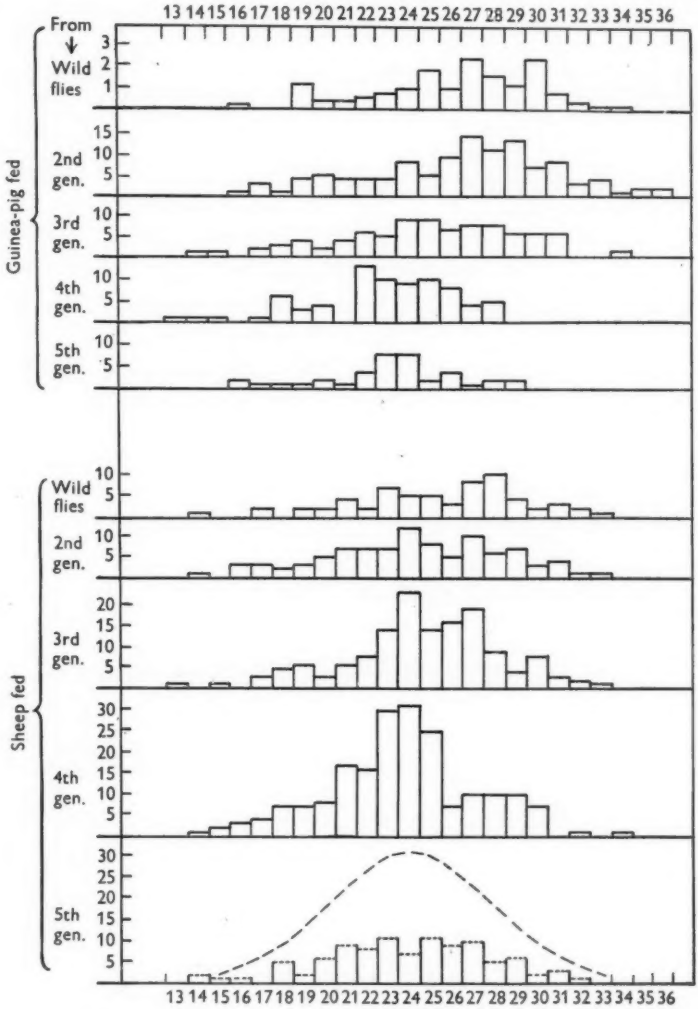

Fig. 2. The distributions of initial weights of pupae obtained from successive generations of *G. swynnertoni* fed on guinea-pigs and on sheep. The vertical scale gives the numbers of pupae, in fives, in each weight-range of 'X. 0' to 'X. 9' mg., the value of 'X' being at the head of the column. The dotted line in the bottom histogram represents the expected numbers from this generation plotted as a normal distribution.

Further, in order to ensure fertility of the original flies, only non-teneral females were accepted, and so the selection exercised tended to exclude those flies which could not produce pupae for some time. abortions. This observation also gives some support to the conclusion reached above in the discussion of interlarval periods that captive flies abort a large proportion of larvae as the effect of captivity in

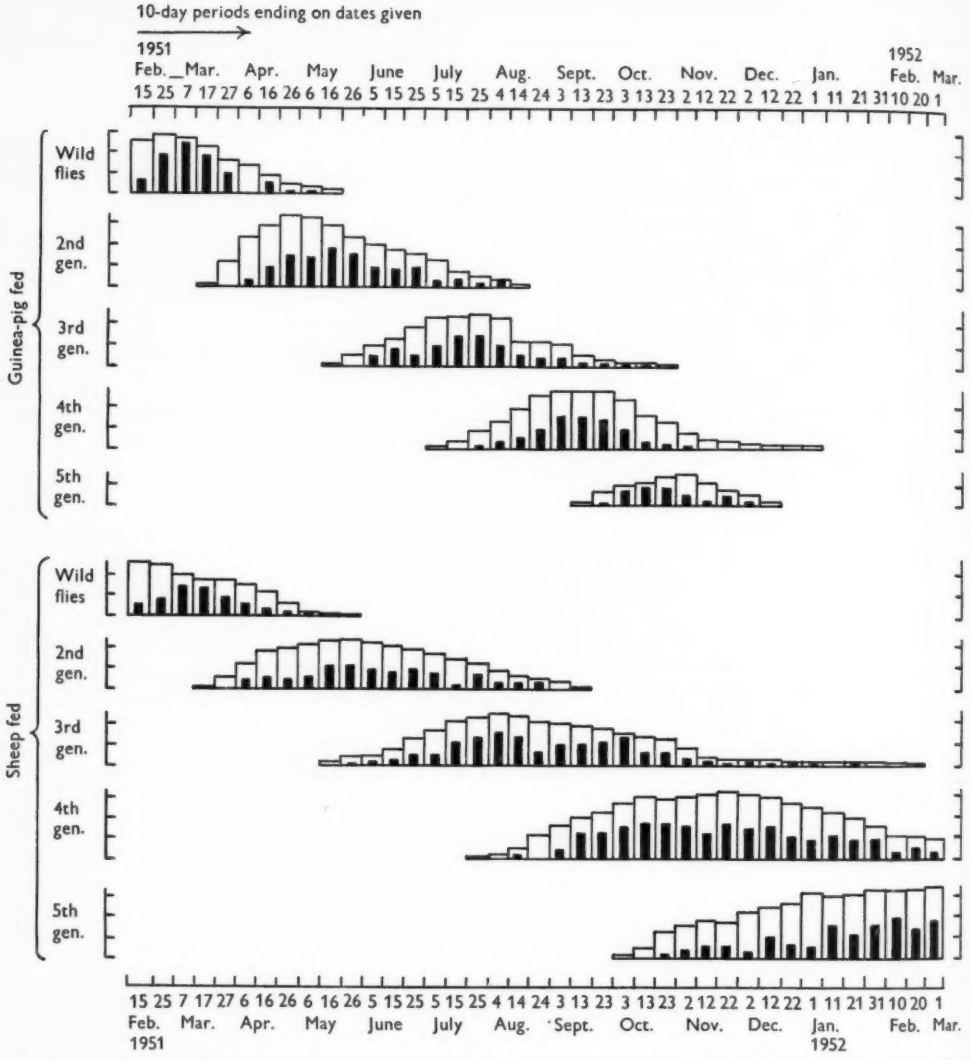
10-day periods ending on dates given
1951
1952
Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.
15 25 7 17 27 6 16 26 6 16 26 5 15 25 5 15 25 4 14 24 3 13 23 3 13 23 2 12 22 2 12 22 1 11 21 31 10 20 1
Guinea-pig fed
Wild flies
2nd gen.
3rd gen.
4th gen.
5th gen.
Sheep fed
Wild flies
2nd gen.
3rd gen.
4th gen.
5th gen.
15 25 7 17 27 6 16 26 6 16 26 5 15 25 5 15 25 4 14 24 3 13 23 3 13 23 2 12 22 2 12 22 1 11 21 31 10 20 1
Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb. Mar.
1951
1952

Fig. 3. The rise and fall of generations of female flies and their succession in time (*G. swynnertoni*). The horizontal scale is divided into equal 10-day periods. The vertical scales are in divisions representing 100 fly-days recorded or a mean of ten flies alive, and the inset columns represent numbers of pupae obtained on the same scale as the mean number of flies alive.

causing abortion might reasonably be expected to be most marked immediately after the capture of a previously wild fly.

The second feature of this figure is that it brings out clearly and quantitatively the different rates of succession of the generations in the two differently fed populations. At first sight it might be thought that the divergence was caused by some obscure difference in the two feeding methods, but closer inspection will show that this is not so. The whole effect can be attributed to two factors, one a chance occurrence and the other a fact which has already

been sufficiently discussed. The first factor is a fortuitous difference in the rate of deposition of pupae in the two halves of the experiment: of the eighty-three pupae from the wild guinea-pig-fed flies sixty were deposited within 30 days, but the sixty-three produced by the sheep-fed flies were much more evenly spread over a longer time. Comparison of the seven pairs of figures for numbers of pupae produced in the first six 10-day periods and after 60 days by a 2×7 χ^2 table gave $\chi^2_6 = 10{\cdot}80$ with a probability of slightly less than 0·1. The discrepancy in rate of production of pupae may therefore be attributed to chance, and the immediate consequence is obvious in the second generation. Here, in the guinea-pig-fed flies, emerging from pupae of which seventy-six out of eighty-three were deposited in 50 days, the population of females rises quickly to a peak within 40 days of the first emergence. In the sheep-fed population, on the other hand, where the pupae were more evenly distributed over some 70 days, the peak of the female second generation numbers is not reached until about 70 days after the first emergence. This effect, once established, will tend to be exaggerated as generation succeeds generation if the mean lives in both populations remain equal. When the second factor is added, namely the longer lives in the sheep-fed population—with the consequent larger number of pupae per fly spread over a longer time—there is clearly no need to seek further explanation of the more rapid succession of the generations in the guinea-pig-fed flies.

Thirdly, the data for the rate of production of pupae are represented diagrammatically in Fig. 3. On the scales used the inset columns for numbers of pupae would be equal in height to those for mean numbers of flies alive if the mean interlarval period were constant at ten days. Similarly, if the interlarval period were 20 days they would be half the corresponding height. The figures given in Table 3, cols. 3 and 7, can be seen to be borne out in these histograms. For example, the long period of 24 days in the second sheep-fed generation is represented by the lowness of the histogram columns of numbers of pupae. (The apparent anomaly of this poor rate of production of pupae followed by the progressive success of the sheep-fed population in later generations is explained by the fact that this generation lasted throughout the coolest months of the year, whereas, in the guinea-pig-fed population, the second generation had passed its highest production-rate before the coolest season and the third generation extended from the tail-end of the cool season into the rising temperatures of the middle dry season). The very poor figures for the fourth guinea-pig-fed generation are reflected in the general low level and early collapse of the histogram of pupa numbers—part of the picture of the population's decline.

Finally, one point should be made as it indicates that these data may lead to a false conclusion. In the results of the orthogonal maintenance experiments given above it was mentioned that the combined survival data of all the experiments gave a significant first-order interaction between maintenance and feeding methods. Searching for a possible explanation of this it was noted that all guinea-pig feeding had been carried out in the laboratory and all sheep feeding outside; in consequence, all cages of flies kept in the laboratory and fed on sheep or kept outside and fed on guinea-pigs had had to be transported some 20 yards immediately before and after feeding, whereas their opposite numbers ('kept in laboratory—fed on guinea-pig' and 'kept outside—fed on sheep') were fed within 5 yards of their place of maintenance. It is therefore just possible that this extra movement, with its resultant disturbance shortly before and after feeding, may have been responsible for the interaction found and for the later decline of the guinea-pig-fed population in the long-term maintenance experiment.

VI. EXPERIMENTS ON THE MAINTENANCE OF PUPAE AND WEIGHT-LOSS DURING THE PUPAL PERIOD

From the first, in the maintenance of pupae, care was taken to minimize disturbance. Pupae were kept singly in $3 \times \frac{1}{2}$ in. tubes with a cotton-wool pad at the bottom and a cotton-wool bung and the tubes were placed on shelves out of the way at the side of the laboratory.

(*a*) *Handling of pupae*

In order to reduce to a minimum the risk of trauma to pupae entailed in frequent weighings, a very simple attachment to a pair of entomological forceps was designed, with which the necessary lifting and moving could safely be done.

The device consisted merely of a small piece of cardboard (of post-card thickness), shaped as in Fig. 4*a*, where it is shown full size, bent at right angles (at the dotted line) and with a small dent pressed into the larger part, the whole being attached to one blade of a pair of entomological forceps with a small pellet of adhesive laboratory wax as in Fig. 4*c*. In its final form the horizontal part was curved up slightly at the front and back to the shape of a shovel (Fig. 4*b*, *c*) and the bend away from the one blade of the forceps reinforced with a little wax below it (Fig. 4*d*) and so adjusted that the other blade passed just above the free edge of the 'shovel' when the forceps were closed. Using this, a pupa can be picked up very readily by sliding the free edge of the 'shovel' under one side of it and gently rolling it into the dent by partially closing the

forceps (Fig. 4*d*). The pupa will remain steady in the depression and only very clumsy movement will dislodge it. To lower it on to another surface such as a balance-pan the forceps are again closed until just touching the pupa, the whole tilted gently with the free edge of the 'shovel' in contact with that surface and the forceps allowed to open slowly, when the pupa will roll off without any fall which might damage the shell (Fig. 4*e*).

alternate days a pupa might have been weighed on days '*E*', '*E*−2', '*E*−4', etc., or days '*E*−1', '*E*−3', '*E*−5', etc.

(*c*) *Conditions and results*

In the earlier experiments the pupae were kept singly in tubes lying on the open laboratory shelf. Later an experiment was performed in which pupae

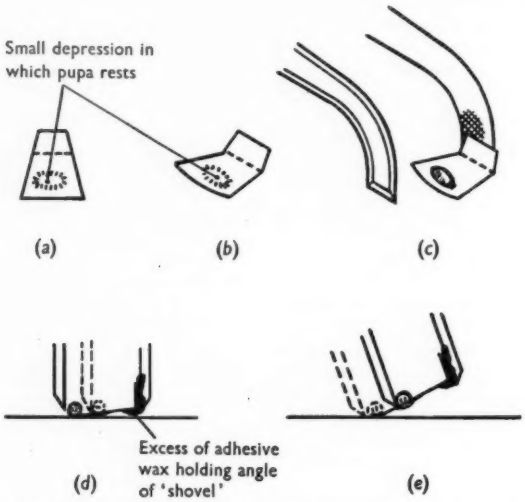

Fig. 4. A simple attachment to a pair of entomological forceps for easy and safe handling of pupae. (Full size). For full description see text.

(*b*) *Data recorded*

Weighings were made on an analytical balance with an illuminated scale showing deviations from the central position and which could be read to 0·1 mg. All *swynnertoni* and *morsitans* pupae were weighed against a 20 mg. weight and *pallidipes* pupae against a 40 mg. weight. As the error of the illuminated scale was less than 0·1 in 20 mg. this method avoided introducing an error of as much as 0·1 mg. in the weighing of even the heaviest or lightest pupa.

At first pupae were weighed daily for ten weighings from deposition and then on alternate days until emergence. Since all pupae were weighed in the morning and emergences occur almost exclusively in the hot part of the day from noon to early evening, a pupa recorded as weighed on the day before the fly was found to have emerged was actually weighed within about 4–10 hr. of emergence. For the purpose of discussion and figures in this paper such weighings will be referred to the day of emergence (day *E*), and, as weighings in this period were on selected at random were kept either in the laboratory or in the outside thatched hut already described. The monthly mean maximum and minimum temperatures under these two conditions are shown in Fig. 5 for one year, and the most marked feature is the much higher minimum temperature inside the laboratory (due to its being shut up at night). So, pupae kept inside the laboratory were exposed to a higher temperature than those outside except for a short period each afternoon when the outside temperature was near its maximum. As, at this stage, no steps were taken to control humidity, the inside pupae were also in a consistently drier atmosphere.

Data from forty-four pupae under each condition of maintenance were collected and examined. Owing to the wide scatter of the initial weights the difference in absolute weight-loss was not significant. The difference between the means of weight-loss only just exceeded its standard error, but the greater loss occurred in the pupae kept inside the laboratory in hotter and drier conditions. When the

losses of weight were expressed as percentages of initial weight the greater loss of the 'inside' pupae was conclusively shown ($t_{86}=4{\cdot}1$, $P<0{\cdot}0001$). During the experiment the effect of the temperature difference on pupal periods was such that for period being only 1·44 % of initial weight. Comparisons were also made of weight loss in pupae at different temperatures by taking the data from those with short pupal periods (at higher temperatures) and comparing them with those from pupae with

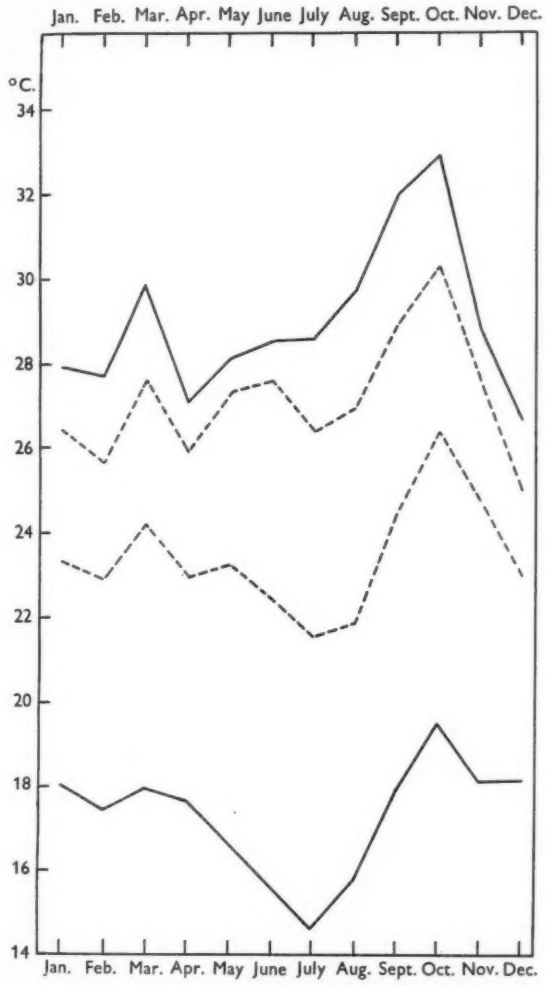

Fig. 5. The ranges of temperature outside (continuous lines) and inside (broken lines) the laboratory during 1951; the points are monthly mean maxima and minima.

'inside' and 'outside' pupae the mean periods were 26·30 and 33·75 days respectively. The mean percentage weight losses from pupation to emergence were: 'inside' 15·65 and 'outside' 14·11, the extra loss in the hotter and drier conditions which produced a difference of 7·45 days in mean pupal long periods under similar conditions of maintenance. As no difference approaching statistical significance was found it was concluded that the effect of temperature on pupal weight loss is trivial in comparison with that of humidity.

Since a short pupal period would have obvious

advantages for laboratory maintenance if the weight-loss could be kept small, it was decided to try keeping pupae at high humidity but at the temperatures inside the laboratory. 90% R.H. was chosen as leaving sufficient margin to make sure that condensation—with the consequent risk of detriment to pupae and interference with weighings—would not occur with any sudden fall in temperature, and as being high enough to ensure that little more weight would be lost than in a saturated atmosphere. The first 115 pupae kept in this way suffered a mean percentage weight-loss of only 11·29, and comparison with the 'outside' pupae above gave the conclusive result of the difference in favour of maintenance at 90% R.H. exceeding its standard error by nearly seven to one.

(1) *Pupal weight-loss and sex-difference found*

With this reduction of total weight-loss there was also a great decrease of the variance of individual observations and, for the first time, a sex-difference was revealed which had previously been concealed in the much larger individual variation. Fig. 6 shows this difference and its development early in the pupal period. Mean percentages of initial weight are plotted for samples of male and female pupae at 1, 2, 3, 4, 5 and 7 days after pupation, for the day of emergence (4–10 hr. before) and for 2 and 4 days earlier. The values for females are shown as '+' and those for males as '⊙', each in the middle of a band representing ± the standard error of that mean.

The data are from forty-eight pupae from which thirty-one female and seventeen male flies emerged. The small numbers are due to a number of restrictions which had to be imposed to get closely comparable data: first, the pupae of the second and later generations have shown a significantly greater weight-loss and greater variances, so, only pupae from the original wild flies could be considered; secondly, of these pupae a proportion either failed to emerge or emerged on one of the days between weighings; and thirdly, only pupae with closely similar pupal periods were included in order to improve the accuracy of comparison. In fact, the thirty-one female and seventeen male pupae were all deposited by captured wild flies, all emerged on the day on which they were last weighed and all had pupal periods of 25–27 days for females and 27–29 days for males. Every male pupa had a period of 2 days longer than the nearest emerging females, and this is shown in Fig. 6 as an extension of the male curve 2 days beyond the female curve.

The general form of weight-loss curves previously published (Buxton & Lewis, 1934; Jack, 1939) is fully confirmed. Weight is lost rapidly for the first 5 days (approximately 20% of the pupal period) and thereafter steadily and much more slowly until just before emergence when, for about 2 days in females and 3–4 days in males, the rate of weight-loss again increases though it never again approaches that of the early period. No points are given for the intermediate period because the data showed a steady loss which is faithfully represented by the interrupted portions of the bands in Fig. 6.

The divergent rates of weight-loss in male and female pupae are clearly shown and the means and their standard errors plotted on the graph are remarkably consistent. The full sex-difference develops in the early period of rapid weight-loss and thereafter the curves are virtually parallel until the final accelerated loss before emergence, when the female pupae lose about 1% of weight in 2 days and the males some 1·5% in 4 days. On all occasions when pupae were maintained at 90% R.H. the difference in weight-loss between sexes was significant, and its value is shown in Table 5 (col. 7) where data for the pupae from the first three generations of *G. swynnertoni* are given.

When it had been determined that 5 days marked the end of the first period of rapid weight-loss, pupae were all weighed at the beginning of the pupal period, after another 5 days and daily for a few days before emergence was expected, in order to get the final weight of every pupa.

(2) *Weight-loss in laboratory-bred generations*

Table 5 shows the means of the final weights of male and female pupae, expressed as percentages of initial weights, for the first three generations of *G. swynnertoni*.

Table 5. *Data on weight-loss of pupae from first three generations of laboratory-maintained* Glossina swynnertoni

	Guinea-pig-fed		Sheep-fed		All		Sex-difference (extra % wt. loss in males)
	♂♂ (1)	♀♀ (2)	♂♂ (3)	♀♀ (4)	♂♂ (5)	♀♀ (6)	(7)
From wild flies	87·85	89·19	87·93	89·33	87·89	89·24	1·35
From 2nd generation	86·09	87·72	85·57	87·14	85·86	87·43	1·57
From 3rd generation	85·08	86·20	85·45	86·57	85·35	86·47	1·12

All figures, except those for the sex-difference, are means of final percentages of initial weight.

The loss of weight became steadily greater from the first generation onwards. In no pair of observations for one sex and generation was there a significant difference between the data from the two feedings, but the difference between male and female pupae within a generation was always significant (first generation, $P<10^{-6}$; second generation, $P<10^{-6}$; third generation, $P<10^{-4}$). The differences between generations in females so, the percentage weight-loss should increase linearly with decrease of size and, with the number of data available from the present experiments, it should have been possible to demonstrate a significant negative linear regression of percentage weight-loss on initial weight. Tests for significance of linear regression were applied to all the six sets of data from two sexes in three generations separately. Only one of these (the second generation females)

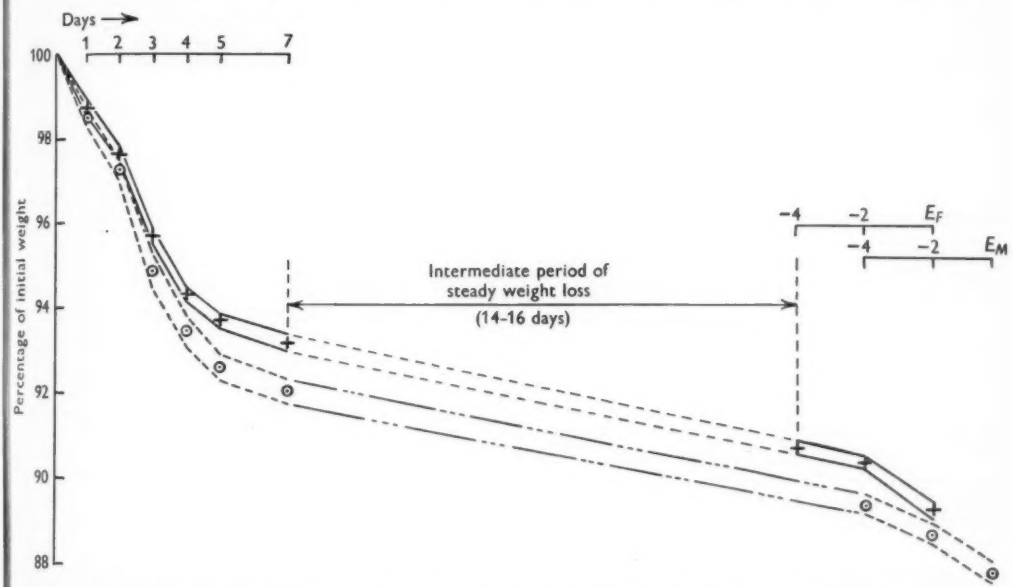

Fig. 6. The weight of male pupae (⊙) and female pupae (+) as mean percentages of initial weight at times from within 24 hr. of pupation to within 10 hr. of emergence. The bands in which the plotted values lie represent ± the standard error of each mean plotted. The ends of the pupal periods are shown as E_F (emergence, females) and E_M (emergence, males) 2 days later, and points are plotted for 2 and 4 days before each of these.

were both significant (first minus second, $P<10^{-9}$; second minus third, $P<0{\cdot}01>0{\cdot}001$); in the males only one was significant (first minus second, $P<10^{-9}$; second minus third, $P<0{\cdot}09>0{\cdot}08$).

With the greater weight-loss in the second and third generations there was also an increase in the variance of observations. The differences in variance were significant only between generations; between opposite sexes and between pupae from the two methods of feeding there was no significant difference in variance.

(3) *Relation of weight-loss to size of pupae*

Finally in the work on pupae, the relationship of weight-loss to size was investigated, as Jack (1939) stated that pupae of *G. morsitans* lost a constant amount of weight irrespective of size. If this were

showed a significant linear regression, the weight-loss increasing about 2% for 10 mg. less in initial weight ($P<0{\cdot}05>0{\cdot}02$). The possibility of non-linear regression was also investigated, and again only one out of six gave a significant result at a probability level of slightly less than 0·01. It was therefore concluded that the data obtained from pupae of *G. swynnertoni* did not confirm Jack's findings with *G. morsitans* and that total weight-loss is in proportion to initial weight.

VII. MAINTENANCE OF *GLOSSINA MORSITANS*

A small experiment was done with *G. morsitans* on the same lines as the main experiment with *G. swynnertoni*, but for this the flies had to be brought

in from over 100 miles away where there was a control fly-round in a *morsitans* area. At the time the experiment was begun only small catches of *G. morsitans* were being obtained, and on one visit only eighteen flies were obtained and only nineteen on another, a month later. The first eighteen were all fed on guinea-pigs and the second nineteen on sheep. Other details of maintenance were the same as in the *G. swynnertoni* experiment.

The main data from this experiment are given in Table 6, and the steady decline of both populations to extinction after the fourth and fifth generations is seen in all data. Only in the third generation of sheep-fed flies was there any approach to a good mean life, with the females living longer than the males and producing a fair number of pupae each, but the numbers are so small that these data are of no value. Further, the seven pupae from this generation were of very low mean weight, only five emerged, one of these emergent flies died very soon and the remaining four, which were all females, did not produce a single pupa.

The initial weights of the pupae from the wild flies showed the same skewness of distribution on the side of underweight pupae as was found with *G. swynnertoni*, the lower limit of normally distributed weights being 24 mg.

As with *G. swynnertoni* all females were examined for insemination after death. All the females of the first and second generations and the two of the sheep-fed third generation were fully inseminated. Of the fifteen in the guinea-pig-fed third and fourth generations, five were fully inseminated, five partially inseminated and five were too far dried to give a reliable result. Therefore, with *G. morsitans* as with *G. swynnertoni*, it appears that failure of insemination cannot be the cause of the poor reproduction rate.

Table 6. *Data from maintenance of* Glossina morsitans

	Sex	No. of flies (1)	Total fly-days (2)	Mean life (days) (3)	No. of pupae (4)	Mean weight (mg.) (5)	No. over 24 mg. (6)	Emergences over 24 mg. (7)
				Fed on guinea-pig				
Wild flies	♀♀	18	651	36·2	27	30·70	24	22
2nd generation	♂♂	10	572	57·2				
	♀♀	9	509	56·6	26	28·66	24	23
3rd generation	♂♂	11	625	56·8				
	♀♀	10	576	57·6	22	26·68	15	15
4th generation	♂♂	6	100	16·7				
	♀♀	5	192	38·4	6	26·48	4	2
5th generation	♂♂	2	90	45·0	—	—	—	—
	♀♀	Nil						
				Fed on sheep				
Wild flies	♀♀	19	936	49·3	26	28·13	19	19
2nd generation	♂♂	11	488	44·4				
	♀♀	9	351	39·0	10	28·28	10	9
3rd generation	♂♂	5	354	70·8				
	♀♀	2	157	78·5	7	25·60	5	5
4th generation	♂♂	Nil	—	—				
	♀♀	4	268+	67·0+	Nil	—	—	—

VIII. MAINTENANCE OF *GLOSSINA PALLIDIPES*

An experiment with *G. pallidipes* was begun on the same scale as the main experiment on *G. swynnertoni*. Sixty non-teneral females were brought in from an area of bush about 14 miles away and put in six cages of ten flies each. The numbers were again divided equally between feedings on guinea-pig and sheep.

The outcome of this experiment may be summarized in two findings which overshadowed all others. Guinea-pig feeding proved entirely unsuitable and no success at all was achieved in getting *G. pallidipes* to mate under conditions of captivity.

These facts are both reflected in the data set out in Tables 7 and 8. The thirty guinea-pig-fed females produced only twenty-three pupae, with a mean interlarval period of 53·85 days. As, on dissection, no flies were found uninseminated this must represent an abortion rate of the order of 75%. From these twenty-three pupae only thirteen flies emerged and their mean life was very short. The showing on sheep feeding was better with seventy-eight pupae,

Table 7. *Longevity data of laboratory-maintained* Glossina pallidipes

		Guinea-pig-fed				Sheep-fed			
	Sex	No. of flies (1)	Total fly-days (2)	Mean life (days) (3)	Longest life (days) (4)	No. of flies (5)	Total fly-days (6)	Mean life (days) (7)	Longest life (days) (8)
Original wild flies	♀♀	30	1575	52·5	108	30	1676	55·9	110
2nd generation	♂♂	6	234	39·0	34–78	23	1057	46·0	123–173
	♀♀	7	135	19·3	22–46	27	1425	52·8	113–188
3rd generation	♂♂		Nil				Nil		
	♀♀		Nil			1	173	173	173

Table 8. *Reproduction data of laboratory-maintained* Glossina pallidipes

	Guinea-pig-fed				Sheep-fed			
	No. of females (1)	Total no. of pupae (2)	Mean interlarval period (days) (3)	No. of pupae per female (4)	No. of females (5)	Total no. of pupae (6)	Mean interlarval period (days) (7)	No. of pupae per female (8)
Original wild flies	30	23	53·85	0·77	30	78	17·75	2·60
2nd generation	7	Nil	—	—	27	1	—	—

fifty emergences and mean lives around 50 days, but even these are not satisfactory results.

Failure to mate under laboratory conditions

From the very beginning of the second generation the difficulty in getting successful matings was such that to find conditions under which *G. pallidipes* would mate became the main preoccupation. Because of the more crepuscular habit of this species, variation of light intensity was tried first. Next, attempts were made in the cool of the morning and evening, both exposed to such light as there then was and in extra shade—this since the thicket habitat of this fly is cooler and darker than its surroundings. The changes were then rung on attempts to mate females, young and old, after their first or later feeds, or between feeds, and all with the previous variables added. In desperation, bearing in mind the findings of Thomson (1948) with mosquitoes, the effect of red, orange and yellow light was tried, but all without success. In all, three matings only were obtained, all with females of the second sheep-fed generation, but in very different conditions. Two of the females died within 2 days of mating and the third produced but one pupa which weighed only 27·3 mg., yet the female fly which emerged from it lived for 173 days.

Fig. 7 (corresponding exactly to Fig. 3 for *G. swynnertoni*) shows the succession of generations and the number of pupae obtained, except that the one third-generation fly is not included.

IX. MAINTENANCE OF *GLOSSINA AUSTENI*

Early in November, 1951, a batch of *G. austeni* pupae was received from Zanzibar by post, and it was decided to attempt the maintenance of this species, chiefly to see what degree of success could be achieved with this fly in conditions very different from those of its natural habitat in the hotter and more humid coastal belt some 4000 ft. lower in altitude.

The very short duration of the experiment makes premature any conclusions on the long-term maintenance of this species, but some results have been obtained that are sufficiently striking, in comparison with those from other species so far tried, to justify a preliminary report.

This experiment differs from those on *G. swynnertoni*, *G. morsitans* and *G. pallidipes* in one respect which should be emphasized in view of the experience of Geigy (1948) that several attempts to start laboratory cultures from pupae all failed. The flies with which this population was started emerged from pupae which had been several days *en route* in the extremely hot, and probably very dry, conditions of a Tanganyika train. When account is also taken of the disturbance caused by excavation of the pupae and their transport by ordinary post—and this in a period which, though externally quiescent, is one of intense physiological activity—the survival of the emergent flies has been truly remarkable.

From the pupae received thirty-six flies, eighteen males and eighteen females, emerged in 24 days. Of the first ten males to emerge and be kept in one cage, five are still alive at the time of writing, 126 days after the first emergence and 120 days after the last addition to this cage. Making the maximum possible deduction for the five dead flies, the mean life of the survivors so far is 121·2 days. Of the eight males that emerged later, two died at about 30 days, two at about 60 days and one each at about 70 and 75 days.

The reproduction data have been equally satisfactory. As only flies emerging from pupae were available, no females could be fertilized until the males had become mature and therefore only a few of the later-emerging females could be mated with mature males after the first feed. Nevertheless, the first pupa was deposited only 26 days after the first female emergence and, so far, seventeen flies have produced eighty-three pupae, one female having died 3 days before the first pupa was found. This number of nearly five pupae per female fly exceeds

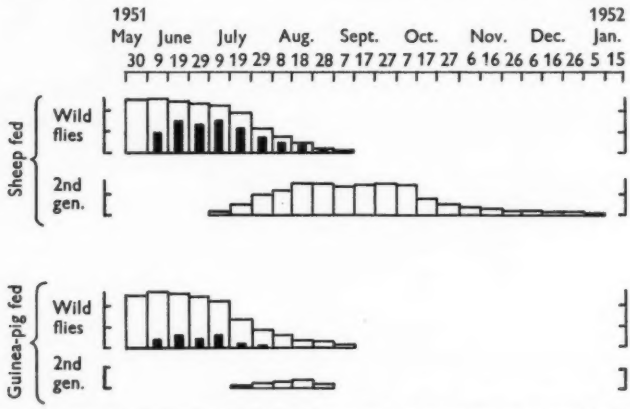
1951
May June July Aug. Sept. Oct. Nov. Dec.
1952
Jan.
30 9 19 29 9 19 29 8 18 28 7 17 27 7 17 27 6 16 26 6 16 26 5 15
Sheep fed
Wild flies
2nd gen.
Guinea-pig fed
Wild flies
2nd gen.

Fig. 7. The succession of generations of *G. pallidipes* on the same scales as used in Fig. 3 (*q.v.*).

The longer life that is expected of females has been fully realized in this species. The eighteen females also were confined in a first cage of ten and a second of eight. In the second cage two flies died at about 25 and 35 days respectively and, since then, a period of 75 days up to the present has passed without another death. Six flies are left alive with a minimum mean life of 115·3 days. The first box of ten females gave by far the best records of longevity that have yet been obtained in any of these experiments; one fly died early at only 13–20 days and there then followed a stretch of 84 days before the next death. With a third death 3 days after the second, there are now seven out of the ten flies alive and their mean life is, at the moment, at least 126·6 days.

The comparison of longevity with *G. swynnertoni* living in their normal climate is very interesting. Only once so far in all the many cages of *G. swynnertoni* kept have ten flies surpassed 1000 fly-days—in one cage of females of the fourth sheep-fed generation—when 1000 fly-days was reached with but two flies alive. In contrast, the first cage of males of *G. austeni* has reached 1000 fly-days—never achieved by males of *G. swynnertoni*—with five out of ten flies still alive, and the first cage of females has reached this point with seven flies surviving.

even the best data from *G. swynnertoni* (Table 3) and, though such a prediction may prove rash, it looks as if this figure will be far exceeded with a final average of over six or even as many as seven pupae per fly. In their rate of production of pupae these laboratory-emerged *G. austeni* are second only to the original wild *G. swynnertoni* fed on guinea-pig and give better figures than any other laboratory-emerged flies; the regression of 'number of pupae' on 'number of fly-days' gives the mean interlarval period as 17·38 days.

Finally, of the eighty-three pupae so far obtained one was a very small larva of 9·9 mg. which died before pupating and the remaining eighty-two have given a mean initial weight of 18·77 mg., individual weights ranging from 12·0 to 25·1 mg. The weight-distribution on sight does not depart significantly from the normal, is very symmetrical, with forty-two pupae above the mean and forty below in quite well-paired groups, and extends for almost equal distances each side of the mean.

One comment on these early results from *G. austeni* may perhaps be permissible as they suggest a possible application of great practical value. If this species is at least as susceptible as others to infection by trypanosomes, as is suggested by the work of Lewis, Wiley & Macaulay (1943), its

remarkable length of life and ability to reproduce, even in a climate very different from that of its natural habitat, should make it a most valuable laboratory vector in the study of problems in trypanosomiasis.

X. SUMMARY

1. Experiments were carried out with the object of devising means of maintaining large numbers of *Glossina* in the laboratory, especially for experimental work in trypanosomiasis.

2. Most of the experiments were done with the local species, *G. swynnertoni*, but maintenance of *G. morsitans*, *G. pallidipes* and *G. austeni* was also attempted.

3. Maintenance at a constant temperature of 26° C. was a complete failure, chiefly owing to the impossibility of maintaining an adequate humidity with the apparatus used.

4. Maintenance in the laboratory (with higher temperatures and consequently lower R.H. for most of the twenty-four hours) gave very poor results and inadequate length of life.

5. Longevity of flies was much improved by keeping them in an open-sided thatched hut in cages over water, and mean lives of up to 110 days in a cage of ten flies were achieved—even over the most severe part of the dry season.

6. Flies were fed in one of two ways: on guinea-pigs in a 'Geigy' feeding-rack or on sheep in a crush by fixing the cages round the flanks and abdomen of the sheep with an elastic band.

7. Earlier comparisons of the two methods of feeding either were inconclusive or favoured guinea-pig feeding. After prolonged maintenance the *G. swynnertoni* fed on sheep gave much the better results. *G. pallidipes*, from the beginning of maintenance, did much better on sheep-feeding.

8. The population of *G. swynnertoni* fed on sheep has increased progressively up to the sixth and seventh laboratory-bred generation; the guinea-pig-fed population showed an early increase but has since declined sharply.

9. Fertilization of females in the laboratory has been easily achieved with *G. swynnertoni* and *G. austeni*. *G. morsitans* has shown some failures, and with *G. pallidipes* great difficulty was encountered.

10. Records of pupal weights and weight-loss have been kept and methods of keeping pupae studied.

11. Results indicated that total weight-loss of pupae was dependent chiefly on humidity and but little, if at all, on temperature; the pupae were therefore finally kept at laboratory temperatures at constant 90 % R.H.

12. In early generations of *G. swynnertoni* and *G. morsitans* there was significant negative skewness of the distribution curves of initial weights of pupae, indicating an excess of small or 'runt' pupae. Flies emerging from these under-weight pupae were eliminated from the maintained population; the lower limits of initial pupal weight, below which the emergent flies were excluded, were 22 mg. for *G. swynnertoni* and 24 mg. for *G. morsitans*.

13. In general, there has been a decrease in the mean weight of pupae from generation to generation, except that the course of the decrease has been very different in the two differently fed populations. In the guinea-pig-fed population weights were high in the early generations and later dropped sharply; in the sheep-fed population weights were rather lower at first but have decreased only slightly and then, in the latest generations, increased a little.

14. There has been an increase in the total weight-loss of pupae from pupation to emergence in later generations.

15. The mean interlarval period has been consistently longer than the known period for *Glossina* at the temperatures experienced, indicating a proportion of abortions up to as much as 50 %.

16. Total weight-loss of pupae was found not to be a constant amount as has been stated elsewhere but to be in proportion to pupal size.

17. A consistent sex-difference in total pupal weight-loss was found in the pupae of *G. swynnertoni* examined, the males losing 1·1–1·6 % more of their initial weight. Data are given to show that this difference is fully established within the early period of rapid weight-loss and only increased a little by the greater loss in the males in the last few days before emergence.

18. The early results of maintenance of *G. austeni* have shown remarkable longevity and very good reproduction-rate, both exceeding the records of other species maintained.

19. It is suggested that *G. austeni* may prove to be a valuable laboratory vector of trypanosomes in view of these promising results in a climate very different from that of its natural habitat.

This work was done at the laboratories of the Tsetse Research Division of the East African Tsetse and Trypanosomiasis Research and Reclamation Organization, and I have first to thank the Director of this Organization for permission to publish this paper. I have called heavily on the long experience and willing helpfulness of both Dr C. H. N. Jackson, now Chief Entomologist of this Division, and Mr W. H. Potts who has only recently retired from the same post, and I am deeply indebted to them both for much advice and encouragement. I also wish to express my thanks to Mr G. R. Jewell, Field Assistant, for his careful and painstaking preparation of the figures and for his valuable contribution in the collection of the wild *G. pallidipes*.

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THE BIOLOGY OF *ORNITHODOROS GRAINGERI* HEISCH & GUGGISBERG, 1952

By R. B. HEISCH, M.D. (Cantab.) and A. E. C. HARVEY

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The tick, recently described by Heisch & Guggisberg (1953), occurs in large numbers in certain caves on the Kenya coast, south of Mombasa. The microclimate of the caves is remarkably constant, the temperature varying from 24 to 27° C. with a humidity of 90% or more. Ten adult female ticks, from a batch collected from one of the caves, were fed on 22 March 1951, and placed in an incubator at 28° C., relative humidity approximately 95%. The first eggs were laid on 3 April.

METHODS

All stages were fed once only on the shaven pinna of a rabbit after the method of Jobling (1924–5), and kept in glass tubes with voile caps, containing small pieces of blotting-paper, at a temperature of 28° C. and a humidity between 95% and saturation.

OBSERVATIONS

The eggs. These were at first a pale amber, darkening in colour after a few days. Of 1091 eggs laid by the original ten females only 457 (42%) hatched compared with 2254 (82%) of 2754 laid by females bred during the experiments. The average period of development was 15 days, varying from 13 to 18.

The larva. The average period between emergence and willingness to attach to the rabbit was 3 days, varying from 1 to 9 days. Although some larvae fed as early as the first day, the majority would not feed before the fifth day. Observations were made on 265 larvae. The time attached whilst feeding averaged 45 min., but varied from 15 to 250 min. The larvae were very active and crawled up the sides of the tubes in which they were contained.

The nymphal stages. The first three nymphal stages were usually able to feed 2–3 days after emergence from the larval or previous nymphal stage; a few, however, fed as early as the first day. Detailed periods were as shown in Table 1. Only

Table 1

Stage	Average period in days	Range	No. of observations
First	2	1–4	203
Second	2	1–8	213
Third	3	1–14	195

Table 2

Stage	Average period in days	Range in days	No. of ticks	Adults produced
First	13	9–27	215	—
Second	18	12–52	197	—
Third	26	13–79	168	82♂♂, 55♀♀
Fourth	25	16–56	15	5♂♂, 10♀♀

a few observations were made on fourth-stage nymphs, the range being from 1 to 24 days.

The periods between feeding and moulting of the nymphs were as shown in Table 2.

The time, to the nearest 5 min., each nymphal stage remained attached while feeding is shown in Table 3.

Table 3

Stage	Average time in minutes	Range	No. of ticks observed
First	30	15–110	189
Second	30	15– 95	201
Third	35	15–100	197
Fourth	30	15– 75	21

Adults. From 168 third-stage nymphs 82 males and 55 females emerged, the corresponding emergence rates for 15 fourth-stage nymphs being 5 males and 10 females. Mating was observed on six occasions, and occurred about 5–10 min. after fed males and females were placed in the same tube. The male first mounted the female from in front, then crawled along its back, finally passing round the posterior border to come underneath, both ventral surfaces being in contact. The first and second pairs of legs of the male were firmly gripped round the second and third pairs of legs of the female. Impregnation

was usually completed after 15 min. All the ticks observed mating had been fed previously. However, on one occasion two ticks apparently mated before receiving a blood meal, for of 30 females selected from a mixed batch of recently moulted adults, one laid eggs after being fed. The males and females of this batch were only together for 24 hr. after emergence from third-stage nymphs, before being separated.

Twenty-four females were observed between mating and oviposition. They laid eggs from 7 to 13 days after copulation.

Fertilized females usually laid rather small batches of eggs over a prolonged period. Although the batches were usually laid daily there were often intervals of several days when no laying occurred. A daily batch averaged 5–6 eggs, but varied from 1 to 20. A fertilized female laid on an average 125 (40–174) eggs over a period of 22 (14–46) days. Four females, fertilized 43 days after a blood meal, laid eggs without being re-fed. The average for these was 103 eggs in 22 days.

Observations at different humidities. A few observations were made with larvae and adults kept at different humidities (temperature 28° C.). These are included here for they demonstrate the sensitivity of the species to microclimatic changes.

Of ten wild caught females kept at a relative humidity of 45–50 %, none had laid eggs after 19 days. On refeeding five were kept at 60–65 %; only two laid eggs (12, 14). The other five were left at 45–50 %, and only one laid eggs. Ten more wild females kept at 60–65 % did not lay during 20 days. After refeeding five were placed at 95 % to saturation; all laid eggs (13, 39, 75, 94, 109). Of the other five left at 60–65 % only one laid.

Forty-nine larvae, fed and kept at a relative humidity of 60–65 %, were all dead within 10 days, and none moulted. A number fed one afternoon and placed in an incorrectly adjusted incubator, the temperature of which rose to 35° C. during the night, were all dead the next morning.

Effects of bite. Several larvae and one female adult were fed on ourselves. In one of us (R. B. H.) the larvae caused temporary itching with slight erythema. In the other (A. E. C. H.) the reaction was more marked the itching continuing for several days with the formation of small papules. The adult tick caused no reaction when fed on R. B. H., but there was itching at the site of the bite immediately afterwards, and a papule developed which itched almost continuously for several weeks and then faded away.

Table 4

Stage	Average period in days	Range in days	No. of ticks	Adults produced
Egg	15	13–18	—	—
Larval	13	9–28	254	—
First nymphal	15	10–31	215	—
Second nymphal	20	13–60	197	—
Third nymphal	29	14–93	168	82 ♂♂, 55 ♀♀
Fourth nymphal	25	16–56	15	5 ♂♂, 10 ♀♀
Emergence of female to meal	3	1– 9	18	—
Mating to oviposition	9	7–13	24	—

SUMMARY

The life history of *Ornithodoros graingeri* under laboratory conditions at 28° C. and 95 % humidity, can be summarized as shown in Table 4. These figures, except for the fourth-stage nymphs, include the interval between emergence and willingness to feed.

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ON A SPIROCHAETE ISOLATED FROM *ORNITHODOROS GRAINGERI*

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This paper describes a spirochaete isolated from two batches of *Ornithodoros graingeri*, collected from caves near Tiwi, about 20 miles south of Mombasa. The ticks were fed on the shaved abdomens of white rats. Unusual features of the new spirochaete were the mildness of infections caused in laboratory animals, and its persistence in a human being. Careful and prolonged examination of thick smears from white rats and mice never revealed more than three organisms, but a general paralytic developed a more intense parasitaemia, as many as forty spirochaetes being counted on one occasion. The organism was morphologically indistinguishable from other relapsing fever spirochaetes and averaged 16·9 μ in length.

SUSCEPTIBILITY OF ANIMALS

The spirochaete was maintained with great difficulty in white rats and mice. Virulence was never enhanced by passage, and attempts at transmission often failed.

White rats and mice. These animals were only mildly susceptible, as shown and summarized in Table 1, and as already stated spirochaetes were very scanty even in thick films (stained with Giemsa's solution). The incubation period varied from 2 to 23 days, blood films seldom being positive for more than 2 days at a time; there was usually only one relapse. Several rats were tested for residual brain infections (see Table 2), but there was little evidence of neurotropism, the only positive animal (rat 12621) having spirochaetes in the blood which vitiated the result. In three of the other animals only the blood proved infective, and it is probable that a clearer picture would have been obtained if the rats had been kept several weeks longer before being tested.

Other animals. Four guinea-pigs, one monkey (*Cercopithecus aethiops*), and a young rabbit were found insusceptible.

Man. Twenty-eight ticks were fed on a patient with general paralysis who was observed for several months. Spirochaetes appeared in the blood on the tenth day and were visible until the thirty-fifth day. These were very scanty early in the infection but suddenly increased, forty being counted in a thick smear on the twenty-second day; the number then diminished, varying from 2 to 10 until the end of the initial attack. The blood was negative by the thirty-sixth day, and remained so except for the fleeting appearance of very scanty spirochaetes on the sixty-first day, which perhaps represented an abortive relapse. The temperature was erratic, irregular, and difficult to correlate with the parasitaemia.

DISCUSSION

The spirochaete behaved quite differently from other strains studied in this laboratory in recent years. It somewhat resembles the Russian rodent spirochaete, *Spirochaeta latychevi*, originally described by Sofiev (1941), and more recently investigated by Baltazard (Personal communication), who noted similar mild infections in laboratory animals, parasites being particularly scanty during relapses. Man, however, was refractory to infection with *S. latychevi*, and ten general paralytics remained negative with normal temperatures, after being bitten by several infective batches of the vector tick, *Ornithodoros tartakovskyi*. The curious persistence of the Kenya spirochaete in the blood of a general paralytic seems unique, and quite unlike the usual course of relapsing fever infections.

Another peculiarity of the new spirochaete was its slight neurotropic tendency, for most tick-transmitted strains cause residual brain infections in a high proportion of white rats and mice. The work of Baltazard suggests that *Spirochaeta latychevi* may also be only slightly neurotropic, for in spite of frequent attempts, it was never recovered from the brains of merions (*Meriones libycus*), regarded as the most important natural reservoir, even when the animals tested were from burrows infested with *Ornithodoros tartakovskyi*. The spirochaete isolated from *O. graingeri* appears to be a distinct species, which, although perhaps related to *Spirochaeta latychevi*, can easily be differentiated from most other tick-borne strains. The animal host has not yet been identified, but the porcupine is suspected. I have pleasure in naming the organism *Spirochaeta graingeri* n.sp. in honour of Mr W. E. Grainger.

Table 1. *Results of inoculating spirochaete into white rats and mice*

Serial no.	Animal	Origin	Daily blood examinations 2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Remarks
12621	Rat	*O. graingeri*	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	Positive on 23rd and 24th days
12788	Rat	Rat brain	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	Blood inoc. into R12871 on 12th day
12784	Rat	Rat blood	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	
12931	Rat	*O. graingeri*	–	–	–	–	–	+	+	–	–	–	+	Sacrificed										
13152	Rat	Rat blood	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
13279	Rat	Rat blood	–	–	–	+	+	–	–	–	Died													
12783	Mouse	Rat blood	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
12954	Mouse	Rat blood	–	+	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Relapse (1 day) on 27th day
12955	Mouse	Rat blood	–	+	+	Sacrificed																		
13112	Mouse	Rat blood	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Positive on 24th and 25th days

Table 2. *Tests for residual brain infections*

Serial no.	Species	Date first inoculated	Date examined	Results of inoculating blood into 'clean' animals	Results of inoculating emulsified brain
12621	Rat	12. x. 51	16. xi. 51	Positive	Positive
12784	Rat	16. xi. 51	27. xii. 51	Negative	Negative
12788	Rat	16. xi. 51	12. xii. 51	Positive	Negative
12931	Rat	8. xii. 51	14. i. 52	Positive	Negative
13152	Rat	14. i. 52	10. iii. 52	Positive	Negative

SUMMARY

A spirochaete, *S. graingeri* n.sp., was isolated from batches of *Ornithodoros graingeri* from caves near Tiwi, south of Mombasa. Very scanty infections were caused in white rats and mice, and a persistent parasitaemia in a general paralytic. Guinea-pigs, a monkey, and a young rabbit were insusceptible. There was evidence that unlike most tick-borne strains the spirochaete was only slightly neurotropic.

I have to thank the Director of Medical Services, Kenya, for permission to publish this paper. I am also indebted to Mr A. E. C. Harvey for help with the experiments.

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ON *ORNITHODOROS ZUMPTI* N.SP., A NEW TICK FROM SOUTH AFRICA

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(With 10 Figures in the Text)

The purpose of this paper is to describe a new tick of the genus *Ornithodoros* Koch, from South Africa. Specimens were received through the courtesy of Dr Zumpt of the South African Institute of Medical Research, after being collected from the burrow of a striped mouse, *Rhabdomys pumilio* (Sparrman), near Cathcart, Cape Province, in April 1952. Interest in the 'burrow haunting' *Ornithodoros* of Africa has increased in recent years, because certain of these ticks have been found to transmit spirochaetes of rodents and other small mammals. These spirochaetes may be evolutionary precursors of those that infect man, and cause the well-known sporadic relapsing fever of tropical Africa. Two live *Ornithodoros* larvae from the burrow were emulsified and inoculated into a white mouse but no spirochaete was isolated (Zumpt, personal communication).

Ornithodoros zumpti n.sp. (Figs. 1–10)

Female. Colour dark brown (dead specimens). Size varying from 3·8 × 2·0 to 4·0 × 2·3 mm. (partially engorged specimens). No eyes. Body with sides almost parallel, bulging slightly opposite coxae II; anterior extremity blunt, and bluntly rounded posteriorly. Integument with numerous round mammillae, the centres having a glistening pearly appearance (for details of dorsal integument see Fig. 2). Dorsal disks present (for arrangement see Fig. 2). *Venter.* Camerostome with lateral 'cheeks' (Fig. 3). Basis capituli broader than long with four short hairs on either side. Hypostome notched, 190 μ in length; four rows of small denticles distally, followed by one row of medium teeth 5/5, and stout teeth 2/2; number of teeth increasing to 3/3 basally, then decreasing to 2/2, 1/1 and diminishing in size. Two slightly serrated post-hypostomal hairs. Palps; articles 1–4 with stout hairs, measuring respectively 180, 80, 70, 100 μ (details of palps and hypostome are shown in Fig. 5). Genital orifice triangular in shape, opposite coxae II, with raised margins. Anus oval and rather prominent (190 × 150 μ); valves with one pair of hairs anteriorly and two pairs posteriorly (Fig. 6). Pre-anal groove shallow, except for central part in front of anus, which consists of an elongate depression lined with elongate mammillae; outer parts of groove broader and triangular in shape. Transverse post-anal groove clearly defined; bisected by median post-anal groove half way between anterior margin of anus and posterior margin of body. Inner part of each half of transverse post-anal groove with smooth unmammillated lip-shaped margins; outer parts of groove ill-defined and mammillated. Median post-anal groove broad, shallow and hourglass-shaped, with two indefinite oval pits placed obliquely behind small transverse mammillated ridge posterior to anus. Supracoxal folds unmammillated, bending inwards opposite coxae IV. Coxal folds slightly divergent anteriorly, bulging inwards opposite depression in front of anus, and extending backwards and outwards to end almost in line with coxae IV. Anterior ends of coxal folds somewhat pointed and opposite coxae II. Spiracles oval, 90 μ across, obliquely placed opposite coxae IV. Legs yellowish in dead specimens; 2nd and 3rd pairs shorter than 1st and 4th. Coxae decreasing in size from I to IV; coxa I slightly separated from coxa II, the rest contiguous; scanty, stumpy hairs on coxal articulations. Tarsi (Figs. 7–10) smooth, rather stumpy, without humps. Length of tarsus I, 0·46 mm.; metatarsus, 0·35 mm. Length of tarsus IV, 0·5 mm.; metatarsus, 0·37 mm.

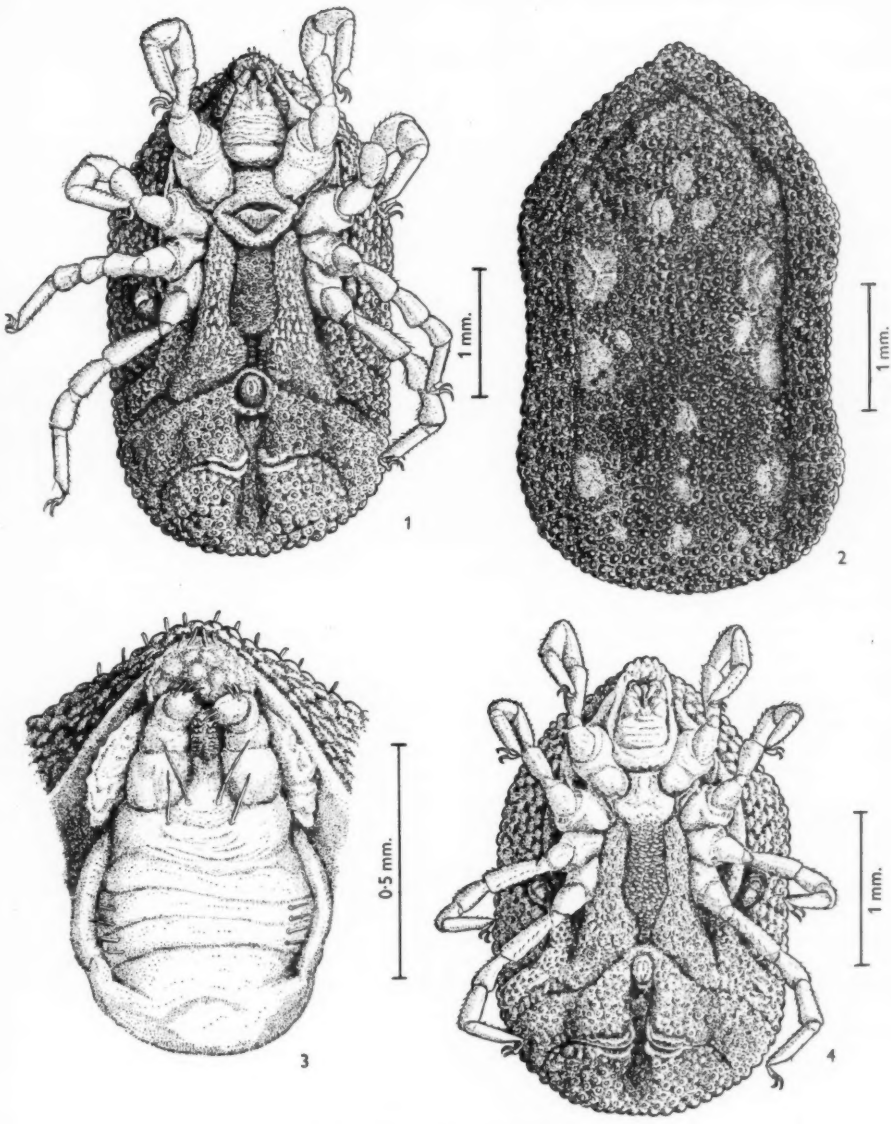
Male. Unknown.

Nymph. The single specimen examined has a shallow depression at the site of the genital orifice and well-developed mammillae; it is, therefore, probably a late stage nymph (see Fig. 4). It measures 3·0 × 2·0 mm. Other characteristics similar to adult.

Described from holotype, and three paratypes (2 females, 1 nymph). One larva mounted on a slide was unfortunately lost in transit. The holotype has been presented to the British Museum and two of the paratypes (1 female, 1 nymph) to the South African Institute of Medical Research.

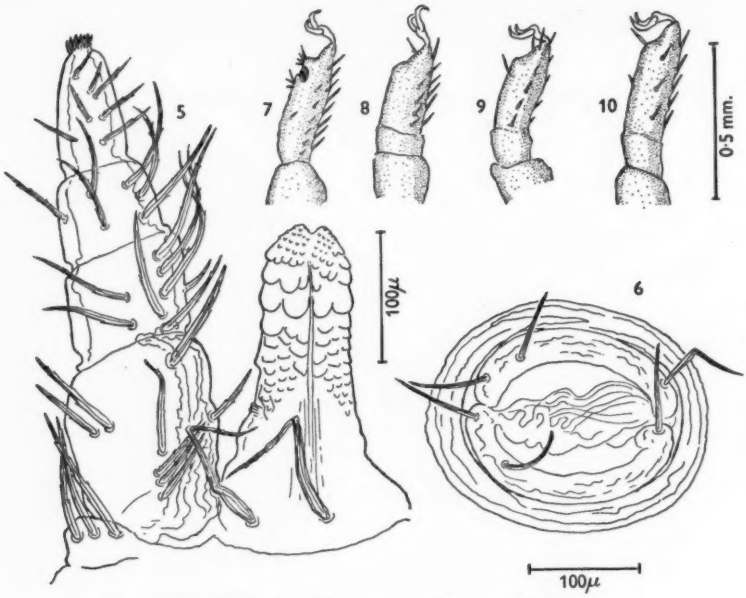
We have much pleasure in naming the tick *Ornithodoros zumpti* n.sp. after Dr F. Zumpt of the South African Institute of Medical Research.

Host. Probably the striped mouse, *Rhabdomys pumilio* (Sparrman).



Figs. 1–4. *Ornithodoros zumpti* n.sp.

Fig. 1. Female, ventral aspect. Fig. 2. Female, dorsal aspect. Fig. 3. Female, camerostome. Fig. 4. Nymph, ventral aspect.



Figs. 5–10. *Ornithodoros zumpti* n.sp. Female.

Fig. 5. Hypostome and palps. Fig. 6. Anus. Fig. 7. Tarsus I. Fig. 8. Tarsus II. Fig. 9. Tarsus III. Fig. 10. Tarsus IV. Figs. 7–10. Camera lucida drawings.

DISCUSSION

The only other tick of the genus *Ornithodoros* known to occur in small burrows in Africa south of the Sahara is *O. erraticus* (Lucas), recently recorded from Kenya and Uganda (Heisch & Grainger, 1950; Heisch, 1952). *O. erraticus*, which inhabits the burrows of pygmy gerbils (*Dipodillus* sp.) on Crescent Island in Lake Naivasha, has been described in detail (Heisch & Guggisberg, 1952*a*), and is easily distinguished from *Ornithodoros zumpti* by its characteristic camerostome with finger-shaped processes. Superficially *O. zumpti* somewhat resembles *O. graingeri* a new tick from caves in Kenya (Heisch & Guggisberg, 1953). Both species have similar coxal folds bulging inwards opposite a pre-anal groove lined with elongate mammillae, and 'cheeks' on the lateral margins of the camerostome, but the post-anal grooves are dissimilar and it is doubtful whether the ticks are closely related. *O. zumpti* was also compared and found different from *O. normandi*, a 'burrow haunting' species from North Africa, specimens of which were kindly provided by Dr Baltazard of the Pasteur Institute, Teheran, and Mr Browning of the British Museum.

We have to thank the Director of Medical Services, Kenya, for permission to publish this paper.

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OBSERVATIONS ON THE RECIPROCAL IMMUNITY BETWEEN SOME AVIAN PLASMODIA

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For the purpose of determining the suitability of malaria for some work on experimental epidemiology, it was necessary to study the reciprocal immunity produced between a number of species and strains of avian plasmodia maintained in canaries. Some of this work has been a repetition of that previously described by other authors, but some new observations have been made, involving a number of geographically widely separated species and strains, which seem worth putting on record.

After an initial patent stage, infections with avian plasmodia persist for a very long time in a chronic stage, so that tests of acquired immunity are strictly tests of the efficacy of premunition against homologous or heterologous superinfection.

The plasmodia used in the present investigation, with their chief differential characteristics, were:

(1) *Plasmodium relictum*, an Algerian strain maintained for some 20 years in canaries at the Molteno Institute, Cambridge, whence it was obtained by the kindness of Dr P. Tate. This, henceforth called 'A' strain, produced a moderately severe parasitaemia during the initial patent period (up to 1000 parasites per 10,000 red blood cells (r.b.c.) at the crisis); mosquitoes (*Culex molestus*) could readily be infected with it.

(2) *P. relictum*, a German (Roehl) strain (similarly maintained at the Molteno Institute), which will be called the 'G' strain, produced a moderate initial parasitaemia (up to 600 parasites per 10,000 r.b.c. at the crisis); in a limited number of attempts it was possible to infect *C. molestus* at a low rate.

(3) *P. relictum*, a Parisian strain recently isolated from a Paris house-sparrow by Dr J. Schneider (of the Pasteur Institute), from whom it was kindly obtained. This 'P' strain produced a severe initial parasitaemia (up to 1500 parasites per 10,000 r.b.c. at the crisis) and *C. molestus* could readily be infected.

(4) *P. relictum*, an Italian strain, kindly sent by Prof. Raffaele. This 'I' strain produced the most severe initial parasitaemia (up to 2000 parasites per 10,000 r.b.c. at crisis) with occasional fatalities, and *C. molestus* could readily be infected.

(5) *P. relictum* var. *matutinum*, kindly sent from America by Prof. Manwell of Syracuse University. Infections produced by blood inoculation with this 'M' strain were uncertain and irregular, but the initial parasitaemia was never more than moderate (up to 1000 parasites per 10,000 r.b.c. at crisis) and always short-lived. The characteristics of this strain have been described by Manwell (1940). *C. molestus* was successfully infected at a low rate.

Except for the 'M' it was impossible to distinguish morphologically between these strains of *P. relictum* with any degree of certainty. Differences in virulence for canaries, as judged by the duration and severity of the initial patent phase of the infection, can be placed in the following descending order: 'I', 'P', 'A', 'G', 'M'. Obvious relapses, short-term and long-term, were seen only with the 'I' strain and were commoner in sporozoite-induced infections.

(6) *P. elongatum*, an Italian strain, also kindly sent by Prof. Raffaele. Infection with this strain was almost invariably fatal to canaries, the birds dying from a progressive anaemia due to the disturbance to the haemopoietic system, although a high peripheral parasitaemia was only occasionally seen. This strain seemed to have lost the ability to produce gametocytes, so that mosquito infection was not attempted.

(7) *P. hexamerium*, an American strain, isolated in 1947, also kindly sent by Prof. Manwell, who has recently described its exoerythrocytic stages as occurring in capillary endothelium and resembling those of *P. relictum* and many other species (Manwell, 1951). This strain produced its characteristic low-grade infection, with a long pre-patent period and frequent relapses. The maximum parasitaemia observed was 600 parasites per 10,000 r.b.c. Although gametocytes were often seen, it was only possible to infect *C. molestus* at a very low rate—one mosquito showing an oocyst out of a total of forty-two dissected from a number of batches which had fed on different birds.

(8) *P. rouxi*, an Egyptian strain, kindly provided by Dr A. H. Helmy Mohammed of the Zoology Department, Fuad I University, Cairo. Like *P. hexamerium*, the other small species, *P. rouxi* produced a low-grade infection with a parasitaemia never more than about fifty parasites per 10,000 r.b.c. It could be differentiated morphologically from *P. hexamerium*, chiefly by the finer structure of the

trophozoites and by the fact that the mature schizonts never contained more than four merozoites.

Other morphological and physiological characteristics of the species used are described by Hewitt (1940, 1949), who also gives references to previous studies on the reciprocal immunity produced by these species and many others. Only the work directly involving the species and strains used here will be quoted.

Gingrich (1932) found complete reciprocal immunity between Algerian and German strains of *P. relictum*, probably the same as the 'A' and 'G' strains used here, and three American strains of *P. relictum*, while there was no reciprocal immunity between any of these and an Algerian strain of *P. rouxi*.

Manwell (1938) found that a latent infection with an American strain of *P. relictum* gave some protection against *P. hexamerium* but not vice versa, while there was considerable but not complete reciprocal immunity between *P. hexamerium* and *P. rouxi*. Redmond (1939), testing the immune relationships between two American strains of *P. relictum* and a number of strains of the closely related *P. cathemerium*, found that the degree of protection produced by one strain against the others was correlated with their relative virulence, being least powerful with the matinal strain of *P. relictum* used. The problem of strain rather than species cross-immunity was studied also by Manwell & Goldstein (1939), who found that there was some variation in the degree of cross-immunity produced by five strains of *P. circumflexum*. A number of workers, including some of the above, have shown that there is no reciprocal immunity produced between *P. elongatum* and any other species of *Plasmodium* in canaries.

In all the work quoted above the inoculation of infected blood and not mosquito transmission was used as the test of immunity.

METHODS

Female canaries were used in these experiments, the blood of each bird being examined before use in order to exclude as far as possible the presence of any pre-existing infection. In testing for the presence of reciprocal immunity chronic cases were selected which were not showing a relapse, and these were inoculated with infected blood taken from a bird during the height of its patent infection. Except where otherwise stated, the chronic cases had all been initially infected by blood inoculation. The test birds in a series, which consisted of two to four chronic cases and one clean control, each received an intramuscular inoculation of from 10 to 50 million parasites of the same *P. relictum* strain, or a lesser dose of *P. elongatum* or of the smaller species which produce a lower parasitaemia in the donor. Each bird in a series received the same dose of citrated blood, but there was no relation between the doses of different series. The duration of the chronic infections in the test birds was from 1 to 6 months after recovery from the acute patent phase. A few birds were superinfected at an earlier stage of their infections, but in this small series there was no apparent correlation between the duration of infection and the strength of homologous or heterologous acquired immunity. Following inoculation, the blood of each bird was examined by Giemsa-stained smears every 2 or 3 days until about 1 month after the end of the pre-patent period usual for the species. Rough estimations of the parasite densities were made by counting the numbers of red cells infected in samples of 500 to 1000, so as to be able to compare the test infections with those in the controls.

A small number of cross-infections were done with the 'A', 'P' and 'I' strains, using mosquito transmission. Batches of *C. molestus* were infected from gametocyte carriers, and when sporozoites were seen in the salivary glands (after 7–10 days at 75° F.) they were allowed to bite birds with chronic infestations. The gorged mosquitoes were dissected until several were found with heavy salivary gland infections, providing circumstantial evidence that the test bird had been inoculated with sporozoites. No attempt was made to assess the number of infective bites received. In a few cases the remainder of the same batch of mosquitoes was allowed to bite a clean control bird 24 hr. later, as a check on the viability of the sporozoites.

RESULTS

The results of the cross-immunity tests using the inoculation of infected blood are set out in Table 1. In order to conform with previous findings, such as the table published by Manwell (1938), the same notation has been used to express the degree of immunity conferred by the chronic infection. In measuring this a comparison has been made of the intensity of infection of the test bird with that of the control, and not the actual severity of infection in the test bird, since the latter varies with the strain and species of the parasite and with the number of parasites in the inoculum. Thus, + + + (=strong immunity) denotes no parasites seen in the test bird; + + (moderate immunity) denotes a parasitaemia up to about one-third that seen in the control; + (slight immunity) from about one-third to two-thirds; − (no immunity) a parasitaemia from two-thirds to as heavy as that in the control. Where a number of similar crosses have been done the average of the results has been taken.

Table 2 shows the results of experiments using mosquito transmission. Since in these experiments only a small number of controls were used simultaneously, successful inoculation being usually inferred from the dissection of mosquitoes, the infections in the test birds have had to be compared with those produced by mosquito transmission of these strains in uninfected birds in the course of other experiments. Nevertheless, the results seem to be sufficiently clear-cut to warrant inclusion.

DISCUSSION

(*a*) The results of the above experiments show that there is no reciprocal immunity between *P. elongatum* and any of the other species tested, and very little, if any, between the larger species, *P. relictum*, and the smaller species, *P. hexamerium* and *P. rouxi*. Between the latter two species there is some slight reciprocal immunity, although quantitative measurements are difficult, but in the small number of tests done, the results appear to be similar to those obtained by Manwell (1938).

(*b*) There is complete reciprocal immunity between the Old World strains of *P. relictum* using blood inoculation except between the 'P' and 'I' strains. Similar results were obtained when mosquito transmission is used. With the latter route, also, in a small number of tests there is some breakthrough of a sporozoite-induced infection when the 'G' strain is cross-infected with 'A' or 'I'. There is incomplete reciprocal immunity between the American strain and any of the Old World strains, this being most evident when the 'M' strain is cross-infected with the 'A' (blood and mosquito transmission) or the 'P' (blood only done). The 'M' strain is the most distinct, physiologically and morphologically as well as geographically, and this raises again the question of when a strain becomes a species. Immunity to homologous superinfection, tried with blood and mosquito inoculation with the

Table 1. *Immunity to cross-infection and superinfection shown by some avian plasmodia, as tested by blood inoculation*

Type of chronic infection	Type of infection inoculated: 'A'	'G'	'P'	'I'	'M'	*P. elongatum*	*P. hexamerium*	*P. rouxi*
P. relictum 'A'	+++ (1)	+++ (1)*	+++ (2)	+++ (2)	++ (2)	− (2)	+ (2)*	.
'G'	+++ (1)	.	+++ (3)	+++ (3)	++ (3)	− (2)	+ (2)	.
'P'	+++ (1)	+++ (1)	.	++ (3)	++ (3)	− (2)	+ (2)	.
'I'	+++ (1)	.	++ (4)	+++ (1)	++ (3)	− (2)	− (2)	− (1)
'M'	+ (2)	++ (2)	+ (4)	++ (3)	.	− (3)	− (2)	− (1)
P. elongatum	.	.	− (1)	− (2)	− (1)	+ (1)	− (1)	.
P. hexamerium	+ (1)	− (1)	+ (1)	− (2)	+ (2)	+ (3)	.	++ (3)
P. rouxi	.	.	.	.	.	.	+ (2)	.

+++ = strong immunity. + = slight immunity.
++ = moderate immunity. − = no immunity.

Numbers in brackets indicate the numbers of test inoculations done in each case.
* One chronic bird initially infected by mosquito transmission.

Table 2. *Immunity to cross-infection and superinfection using* Culex molestus *to transmit the test infection—'A', 'P' and 'I' strains of* Plasmodium relictum *only.*

Type of chronic infection	Type of infection transmitted by mosquito bite: 'A'	'P'	'I'
P. relictum 'A'	+++ (1)	.	+++ (2)*
'G'	++ (1)	+++ (1)	++ (2)
'P'	+++ (2)	.	++ (3)*
'I'	+++ (2)	++ (1)	+++ (2)*
'M'	+ (2)	.	++ (2)
P. hexamerium	− (2)	.	− (1)

Notation as in Table 1.

'A' and 'I' strains only, is complete. With avian plasmodia it appears that infection with one strain gives considerable protection against cross-infection with a heterologous strain of the species. This is in contrast to the plasmodia of higher animals, notably of man and apes, in which it has been conclusively shown that there may be little reciprocal immunity between strains of a species coming from within comparatively small geographical areas and showing no great difference in physiology and morphology.

I owe many thanks to the staff of the Ross Institute for assistance in maintaining the strains and with the blood examinations, and to Prof. Garnham for much advice.

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GURLEYA SP. (MICROSPORIDIA) FOUND IN THE GUT TISSUE OF *TRACHEA SECALIS* (LEPIDOPTERA)

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(With 15 Figures in the Text)

Among approximately eighty larvae of *Trachea secalis*, gathered from the same locality near Cape Town, South Africa, the incidence of microsporidian infection has been found to be 100%. In the majority of cases the parasite had heavily involved the lower portion of the gut, whereas the rectum and the upper portion of the gut were never infected. In severe cases the gut tissue was practically destroyed and replaced by an almost solid mass of spores. Except during the stages preceding spore formation, the infected portion of tissue can be detected by the unaided eye. When spores have been produced in large numbers, the involved area, which may be no larger than the head of a pin, and is usually located within the posterior third of the gut, becomes white and opaque, and contrasts sharply with the healthy surrounding tissue. The latter is transparent, and appears dark, owing to the colour of the contents. At a later stage it is not unusual to find the infected zone in the form of a narrow band which encircles the gut. The infection then spreads upwards and downwards. Finally, the entire lower portion of the gut becomes white and chalky. With the exception of the gut contents the parasite has not been found elsewhere in the body of the host.

FIRST FORMS

No extracellular planont stage has been observed in the gut contents or elsewhere. During the earliest observed phase of the life cycle of the *Gurleya* a few cells within a small zone of the gut tissue are found to harbour a number of spherical parasites measuring about 3 μ across. Their nuclei are eccentrically situated. These forms never invade the nucleus of the host cell, but are always located within the cytoplasm. They do not lie within clear areas, as has frequently been recorded in other genera, a condition supposedly due to the secretion of a lytic agent (Fig. 1).

According to the commonly employed terminology this phase is classed as schizogony, and the forms as schizonts, but in the present case these terms cannot reasonably be employed, as true schizogony definitely does not occur. Multiplication is by binary fission only; a few multinucleate plasmodia may be encountered, but even in these cases it is by no means certain that cytoplasmic continuity persists, as the bodies might merely be closely packed. No form of motility has been observed among the organisms when studied in the living state.

SPOROGONY

Sporogony begins while the focus of infection is very limited. No evidence has been afforded indicating that fusion of gametes or karyogamy precedes the development of sporonts; many of the previously described forms are in a state of division, and it is difficult, with so many binucleate cells present, to decide whether their nuclei are separating or fusing. The first indication that the organism is going on to sporogony is an increase in the size of the cells to about 4·5 μ in diameter. Nuclear division then takes place, the two daughter-nuclei separating until they occupy opposite poles. The cell then elongates in the direction of the nuclei until its dimensions are about 9 by 3·5 μ, and at the same time the cytoplasm exhibits a changed appearance after staining by Leishman's method; it becomes streaked and patchy, and stains only very delicately. There is often a slight constriction in the middle of the body (Fig. 2). A second nuclear division follows, and two of the nuclei move towards the centre of the cell (Fig. 3). Cytoplasmic protuberances then appear on either side of the middle of the body, each of which becomes occupied by one of the nuclei (Fig. 4). Cytoplasmic cleavage then commences, and progresses until continuity remains only in the central area of the mass (pansporoblast). The four sporoblasts then radiate symmetrically outwards, the nuclei in each case being located at the point farthest from the centre of the group. Occasionally the body becomes only slightly elongated after the first nuclear division, and afterwards becomes rounded. The second division, in such cases, is followed by cytoplasmic fission which results in a similar pansporoblast. There is no ectoplasmic membrane investing the four sporoblasts (Fig. 5), which finally become separated. The sporoblast is a round

cell measuring from 3·5 to 4·5 μ across, its nucleus is eccentrically situated, while the cytoplasm stains evenly, but very delicately, by Leishman's method.

Wet-fixation and staining reveals that the nucleus of all forms of the parasite is vesicular, with a central karyosome, and also that during all stages from the binucleate sporont to the mature pansporoblast the body is invested in a tough ectoplasmic pellicle which maintains its form even when considerable internal cytoplasmic shrinkage is evident (Figs. 6 and 7).

MATURATION OF SPORE

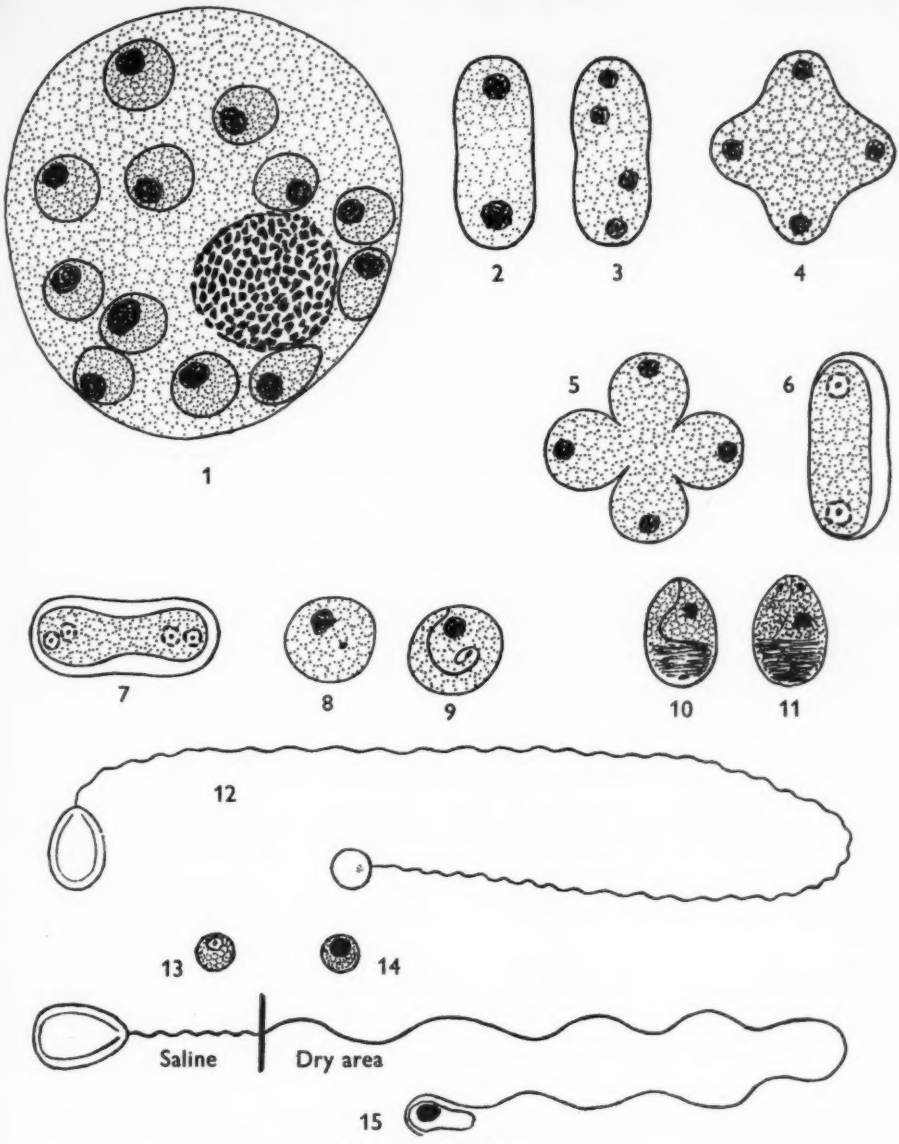
Maturation of the sporoblast does not occur until it becomes separated from its group. First, a small, faintly staining granule of chromatic material appears at a point diametrically opposite to the nucleus (Fig. 8). If this particle originates from the nucleus it must be expelled from it in some unusual manner. No form of unequal division has been observed which might give rise to a second small nucleus. The filament immediately begins to grow from the chromatic granule. It usually encircles the granule once or twice and then strikes out at a tangent and passes, more or less directly, to the border of the cytoplasm at a point near to the nucleus (Fig. 9). The sporoblast then elongates until it measures about 4·3 by 2·6 μ, the end nearest to the nucleus always being the more pointed (Fig. 10). Meanwhile, the development of the coiled filament proceeds rapidly, and soon it occupies the posterior (the more rounded) half of the body. From this point subsequent development becomes difficult to follow owing to the formation of the capsule. During the last stage in which internal detail can be made out the coiled filament occupies the more rounded end, while occasionally two very small chromatic granules can be observed near the anterior end. The sporoplasm nucleus lies between them and the coiled part of the filament (Fig. 11). Thereafter, the capsule is rapidly developed, and the interior of the spore becomes unstainable. The mature sporocyst, which is somewhat smaller than the sporoblast, measures about 3·5 by 2 μ. The sporocysts are found in large numbers in the gut contents, and in the faeces of the host. Sporocysts are also found in the moth, but here the earlier developmental stages are absent.

A small proportion of sporocysts of a larger type are also always present. They measure about 6 by 3 μ and appear to possess internal details similar to those of the smaller sporocysts. They are apparently derived from sporoblasts similar to those giving rise to the smaller sporocysts, but increase in size during maturation. Several authors have stated that the large sporocysts of other genera do not possess a polar filament, but in this case the development of the filament has been observed and studied in the sporoblast. The act of filament extrusion has been observed in the case of the larger sporocysts and empty capsules have been found, in some cases with the extruded filament still attached.

EXTRUSION OF FILAMENT

Filament extrusion has not been found to take place when fresh material is immersed in saline, but a few forms are usually present with the filaments extruded and the capsules empty. Neither the addition of acids nor alkali, or of iodine to the saline has been found to induce filament extrusion in the case of fresh material. It has been found, however, that after the sporocysts have been subjected to a period of drying, the filaments will be extruded almost immediately when they are immersed in normal saline. If the bulk of the infected material is small, a few hours in the dried state will suffice. Sporocysts have been found to be viable, and to extrude their filaments vigorously in saline after 6 weeks of desiccation. Ohshima (1937) states that the haemolymph of silk-worms has no effect on spores of *Nosema bombycis*, but in the present case the haemolymph of the host, although inactive for undried sporocysts, induces more vigorous filament extrusion than normal saline after they have been subjected to drying. Filament extrusion commences within about 1 min. of being immersed in either the haemolymph or saline, and continues vigorously for about 10 min., after which the phenomenon gradually abates.

When the filament is shot out, there is a vigorous reaction upon the cyst, and violent vibratory movements or rotation can be observed. If the filament encounters tissue or cells during the extrusion, these are penetrated. This phenomenon can be observed in the case of sporocysts which have extruded their filaments in the haemolymph of the host. In this case the blood-cells are frequently pierced. The fully extruded filament measures about 60–80 μ in length. Korke (1916) observed minute uninucleate or binucleate amoebula-like bodies attached to the tips of extruded filaments of *N. pulicis*, and further stated: 'The function of the filament has been stated to be the fixation of the spore to the gut epithelial cell; but it may be that it also serves to conduct the sporozoite (amoebula) to a distant part of the tissue and thus to ensure an advance of the parasite into fresh areas.' It would appear that he believed that the filament was extruded with the sporozoite adhering to its tip. Ohshima (1937), who describes a somewhat involved method of inducing filament extrusion in *N. bombycis*, points out that, in that species, the infective body (sporoplasm) appears at the extremity of the filament at the moment of its extrusion, and concludes that it is actually poured from the tip of the filament.



Figs. 1–15. *Gurlea* sp.

Fig. 1. Infected gut-cell during first phase.
Fig. 2. Sporont after first nuclear division.
Fig. 3. Sporont after second nuclear division.
Fig. 4. Quadrinucleate pansporoblast.
Fig. 5. Pansporoblast with four mature sporoblasts.
Fig. 6. Binucleate and (Fig. 7) quadrinucleate sporont, showing tough ectoplasmic pellicle. (Wet-fixed and stained by Ehrlich's haematoxylin.)
Fig. 8. Sporoblast showing chromatic granule.
Fig. 9. Sporoblast showing filament growing from granule.
Fig. 10. Elongate sporoblast showing path of filament.
Fig. 11. Sporoblast as seen just before complete development of capsule.
Fig. 12. Sporocyst with extruded filament, showing ejected sporoplasm adhering to tip. (Fresh state.)
Fig. 13. Sporoplasm after wet-fixation and staining by Ehrlich's haematoxylin.
Fig. 14. Sporoplasm dry-fixed and stained by Leishman's method.
Fig. 15. Sporocyst with filament extruded in saline, showing path of filament over dry area of slide, and ejected sporoplasm.

Note. All figures, with the exception of Figs. 6, 7, 12 and 13, are represented as seen when dry-fixed and stained by Leishman's method.

The same phenomenon can be observed in the present parasite, and evidence has been afforded (*vide infra*) in support of Ohshima's contention. A small globule (the sporoplasm) becomes apparent at the end of the filament immediately it is sufficiently quiescent for observation, while, almost simultaneously with extrusion, the capsule can be observed to have emptied itself (Fig. 12). The sporoplasm is a globular body measuring about 1·75 μ across, and exhibits a single, eccentrically situated nucleus which can be faintly seen in the living state. The globule remains attached to the tip of the filament for some seconds, after which it becomes free and floats away. If extrusion has occurred in normal saline, it soon distintegrates, and it is difficult to obtain stained specimens from such preparations. The disintegration is, apparently, not due solely to osmotic pressure differences, as it occurs even in hypertonic solutions. If, however, the saline is lightly albuminized the sporoplasms maintain their form, and stained preparations reveal them well. They are also well preserved in the case of sporocysts which have reacted to the haemolymph of the host.

In the fresh state, or after wet-fixation and staining, the sporoplasm is always spherical, but dry-fixed specimens sometimes have an appearance suggestive of amoeboid activity; this is doubtless the result of distortion of the fragile body during drying. When wet-fixed and stained by Ehrlich's haematoxylin and eosin the nucleus has a vesicular structure, and possesses a single, central karyosome (Fig. 13). After dry-fixation and staining by Leishman's method the cytoplasm takes on a fairly deep colour, and the nucleus, which occupies more than half the diameter of the cell, is also very strongly stained (Fig. 14).

The study of filament extrusion is aided considerably by the following method of inducing the extruding filaments to pass over a semi-dry surface. A mass of ripe sporocysts, which have been subjected to desiccation, is mounted in saline, under a cover-glass, which is then momentarily lifted and replaced several times in order to form numerous air-pockets. Many small groups will then probably be found to lie within minute droplets of the liquid which are surrounded by air. Under these conditions the sporocysts contained in the droplets will extrude their filaments, but in the case of those situated near the border of the liquid, and which are suitably orientated, the filament will issue for only a small part of its length before it passes out of the saline and into the air-pocket. When this occurs its rate of progress is reduced to such an extent that several seconds are required for it to become fully extended.

There can be no question of its progression, within this area, being the result of the initial velocity with which it leaves the sporocyst. Neither can it result from pressure exerted upon it by that portion of the filament within the liquid since, first, after the initial shock which occurs at the moment of extrusion, there is no further reaction upon the cyst while the filament is slowly moving over the dry area and, secondly, the filament flows in serpentine form, and each wave, once formed, remains unchanged, a circumstance which is not readily reconcilable with posterior pressure. Perhaps the picture of the passage of the filament across the dry area can best be described as being reminiscent of that resulting from the act of drawing a wavy line, with a pencil, across a piece of paper.

At the instant the progression of the filament ceases, but never before, a gush of protoplasm (the sporoplasm) pours from its tip. Since the material is ejected upon a dry surface instead of into a liquid medium, the sporoplasm does not attain the globular form assumed when ejected into saline. Stained preparations show that this body contains one nucleus (Fig. 15). This 'slow-motion' study of filament extrusion in the present parasite, considered in conjunction with the penetrating powers of the filament, bears out Ohshima's contention that the sporoplasm is introduced deeply into the tissue by the filament, and is thus protected from the action of the digestive juices during its passage from the sporocyst.

It appears probable, therefore, that the conjecture of several authors, that the filament consists of a hollow tube which has to be completely everted during its emergence from the spore is correct. Indeed, it is difficult, otherwise, to explain the passage of the filament, as described, over a dry surface. The hypothesis also explains the serpentine-like path of the slowly extruding filament. As the filament was previously in coiled form, it appears likely that, when considered as an everting coiled tube, it would tend to follow a wavy path in liquid, over a dry surface, or even when passing through tissue. The hypothesis also renders the tissue-penetrating powers of the filament more comprehensible.

DISCUSSION

The organism passes on to sporogony very early during the course of the infection, and while the parasitized zone is small. It is difficult, therefore, to explain the subsequent rapid extension of the infection. A possible explanation appears to be that, while the majority of the parasites pass on to sporogony, a certain number remain undifferentiated and continue reproduction by binary fission, thus providing for the parasitization of fresh host-cells. It has been pointed out that a few empty sporocysts are always to be found in fresh material, and it is possible, in view of this fact, that the extension of the infected zone may be assisted

by the release of fresh sporoplasms by a certain proportion of the spores.

Despite the destruction of a large portion of the gut tissue, the organism does not produce any observable pathological condition in the host. As pointed out, infected larvae pass through the metamorphosis, and the spores can be found in the resultant moth.

It has been definitely established that, in the present parasite, the infective body (sporoplasm) is ejected from the free end of the filament at the instant of extrusion, and since the often-made statement to the effect that the sporoplasm creeps out of the sporocyst after filament extrusion is more of a hypothesis than an observation, it appears likely that the described ejection of the sporoplasm from the filament will be observed among other microsporidia. The described organism qualifies for inclusion in the genus *Gurleya* since the pansporoblast consistently produces four sporoblasts, but it is not intended, at this stage, to propose a specific name.

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ON THE BLOOD-SUCKING MIDGE *CULICOIDES VEXANS* STAGER, INCLUDING THE DESCRIPTION OF ITS EGGS AND THE FIRST-STAGE LARVA

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(With 4 Figures in the Text)

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INTRODUCTION

At one of the meetings of the Royal Society of Tropical Medicine and Hygiene, I made a very short communication on the three blood-sucking midges (Jobling, 1929). My particular reference was to *Culicoides vexans*, which is the most troublesome and commonest species in spring, in the northern and the north-western suburbs of London. At that time I had been studying the life history of this midge, including the morphology of its eggs, larvae and the pupa, but this work was not completed.

During the last few years the blood-sucking midges attracted a good deal of attention in the north of England and particularly in Scotland, where one of them, *C. impunctatus* Goetghebuer, is the major pest (Hill, 1947; Macgregor, Cameron, Downes Morison, Peacock & Todd, 1948; Kettle, 1949). Therefore, I think that the results I obtained, although incomplete, may be of interest to all those who are studying the midges, especially in the north as, according to Edwards (1926) and Edwards, Oldroyd & Smart (1939), *C. vexans* is not recorded in the north of England, and its name is also not present in the Survey of Scottish Midges, given by Cameron, Downes, Morison & Peacock (1946).

OCCURRENCE AND HABITS OF THE MIDGE

It was found that *C. vexans* has only one generation a year. It appears in spring about 2 or 3 weeks later than *C. pulicaris* and *C. obsoletus*, usually at the end of April or the beginning of May, but when the first month of spring is mild, it may be found about the middle of April. During the first 2 weeks after appearance, its numbers increase more or less rapidly, and then gradually begin to decline. Near London it completely disappears at the end of June or the beginning of July.

The midge bites even during the day, but usually begins to be very troublesome in the evening, about 2 or 3 hr. before sunset. With the appearance of darkness it gradually disappears, and does not attack at night in the open. However, it enters houses, and on a dark night may gather in large numbers around the electric light on the ceiling, and will then bite all those who happen to be near the source of light. It is especially troublesome on dull sunless days, when it may attack in such a vast swarm that the mass of midges looks like grey smoke. Such swarms were observed on 30 May 1926, and on 20, 22 and 23 May 1927. During these days the weather conditions were respectively as follows:

	Temp. (° C.)	R.H. (%)
Very dull and quiet	+16	85
Very dull and quiet	+14·5	74
Very dull and quiet	+11·5	91
Very light breath	+14	79

As regards the influence of temperature, the midge was found to be very active between +9·5 and 17·5° C. On hot dry days it may be completely absent, even in the evening. During this kind of weather it hides near the roots of grass or in crevices and cracks in the soil, where, when I was searching for eggs, females were found many times, some as deep as 5 in. below the surface of the ground.

Very light drizzling rain has only a slight effect on the midge, provided that there is no wind. During damp sunless weather, which is the most favourable to the midge, it may be completely absent if there is a moderately strong wind. During the day it shows a distinct negative phototaxis by its avoidance of sunshine. Thus on 15 May 1926, very light showers alternated with bright sunny intervals, and every time when the sun was obscured by clouds, numerous females came out to bite. As soon as the sun shone out again they very quickly disappeared. This behaviour of the females was observed three times in succession.

The males of this midge were found several times among the females when the latter were flying near me in the evening. It is quite possible that they do not swarm like those of *C. pulicaris*. The swarming of the latter species was observed many times during the quiet weather in the evening, about 2 ft. from the ground.

People wearing dark clothes are more readily attacked by the midge than those in light-coloured clothes. Hill (1947) successfully used pieces of black cloth in her study of the annual incidence of the other four species of the genus. According to my observations a dark-grey colour is more attractive than black, but on the black surface the midges are more distinct than on the grey surface. The usual site of the attack is the back of the neck and the arms. When the midge is present in very large numbers, it may settle on any part of the face or on the ankles, or may crawl under the collar and up the sleeves. It can easily pass through mosquito netting and bite through thin stockings. In animals such as cats and dogs it attacks the ears and also those parts of the head where the hairs are short and are not very dense. On the human forearm it gorges itself in about 3 min. The effect of its bite has been described by me (1928). It produces intense irritation and usually a very extensive swelling, which very often may last for 3 days, or longer in more susceptible victims. Moreover, in allergic individuals a single bite may produce hypersensitivity which manifests itself by the appearance of itching in the previously punctured spots of the skin. This reaction appears immediately or a little after the bite. According to my own experience, it is of a very short duration, lasting only a few minutes.

DISTRIBUTION AND BREEDING SITE

Near London the midge occurs on London clay. It is absent from many parts of Surrey where the soil is sandy or peaty and from the downs near Epsom and Box Hill where the soil overlying the chalk formations is very porous and has very good drainage. As far as my observations go, it does not occur on every kind of clay. For instance, it is not present near Claygate and Chessington in Surrey. The clay of these two places differs from that of London in that it contains very minute particles of sand. It feels very gritty when rubbed between the fingers, and is of a much darker colour. Everywhere where this midge occurs its distribution is patchy. It is much more numerous along the margins of spinneys and woods, and near clumps of trees and bushes, especially if they are growing in low-lying meadows. In these places the midge is more common on the sheltered side than on that which is exposed to the prevailing wind. The same behaviour in relation to the wind was found in *C. impunctatus* by Kettle (1951*a*, *b*), who investigated more fully the spatial distribution of this species.

The presence of the midge in many places far away from ponds and streams suggested that it breeds in the soil. Therefore, in order to find its breeding site, several samples of soil, measuring $2\frac{1}{2} \times 2\frac{1}{2} \times 2$ in. each, were collected on 1 May, from different places between Stanmore and Edgware. These samples were brought to the laboratory, where each of them was put into a separate Petri dish containing a little water to keep the soil moist. The dishes were kept under lamp chimneys of which the upper end was covered with muslin.

The sites from which the samples were taken and the number of *C. vexans* which emerged from them were as follows:

Shady ground, about 8 ft. from trees, partly under branches, covered with water in winter and spring. Soil almost pure clay of light colour, deficient of humus and with a few patches of very short grass. Five females and two males.

Situation as above, waterlogged in winter. Soil very poor clay loam, supporting patches of short grass, a few buttercups and dandelions. Three females.

Low-lying ground, partly under branches of trees, waterlogged in winter. Very heavy soil of light colour, supporting a few patches of very short grass. Four females and one male.

No *C. vexans* appeared from the following samples: clay loam of very dark colour, about 1 ft. from a large oak tree; humus-containing soil from a spinney; humus-containing soil from under a bush; two samples of turfy loam from a meadow; three very large samples of the cultivated garden soil; cow dung-cakes; and two large collections of debris from two tree-holes.

Although the number of samples was small, and they were taken when there were already a few midges about, these results show that the breeding sites of this species are waterlogged patches of ground, situated in more or less shady places, with poor clay soil, which supports only very sparse low-growing vegetation.

RELATION TO DOMESTIC ANIMALS

In the spring of 1928 the midge became very rare. No swarms were observed on dull days, and only a very few specimens were obtained in those places where before it was possible to collect hundreds in a very short time. This remarkable drop in the midge population occurred after the removal of a herd of cows from the site, when the latter was acquired by the local authorities for a recreation ground. Continued observation on this site during the next 4 years revealed no increase in the number of midges. During those 4 years no changes were made to the site, except for the removal of a few very old trees, so that the breeding conditions in the soil were not modified, and therefore not accountable for the reduction of the midge population.

The site has been visited in May twice since the war, and although during each visit almost the whole evening was devoted to collecting, the total catch consisted of only five specimens. During the last 2 years similar attempts have been made on my behalf by Mr P. L. Bradley, a former laboratory assistant, with equal lack of success. His largest catch consisted of six specimens. Further, no midges were obtained from eight samples of the soil collected by him from the same patches of the ground as those described in the preceding section.

Although since its opening the recreation ground has been frequented by large numbers of people, the midge population has remained very much smaller than when this site was used as a grazing ground. These observations show that such animals as cows are very important hosts, and capable of maintaining a very much larger population of this midge in its breeding area than are man and small domestic and wild animals.

OVIPOSITION

Like other blood-sucking Diptera, the females of this species are fertilized before feeding. Out of more than thirty dissected females, which were caught on the forearm, only two were found without spermatozoa in their spermathecae. The observations of Hill (1947) and Parker (1950) show that the females may take more than one blood-meal before oviposition. Parker found that *C. pulicaris* and midges of *C. obsoletus* group can feed after laying the first batch of eggs, and then lay the second batch. These authors showed that in the species studied by them, the number of eggs in the batches varies considerably.

As all my attempts to find the eggs of *C. vexans* were unsuccessful, I decided to use the females which were fed on the forearm in the open, and see if they could lay the eggs in the laboratory. On 9 June six fed females were introduced under a glass chimney, which was standing on a Petri dish filled with moist soil from the breeding site. On the surface of the soil was arranged a very flat piece of stone, which was supported by three small stones, in such a way that there was a gap about three-quarters of an inch between the flat stone and the surface of the soil in the dish. It was thought that this arrangement would provide the females with conditions more or less similar to those which exist in the soil of their breeding site.

A few hours after the introduction of the females under the glass chimney, they all went under the flat stone, and during the next 6 days never appeared outside. On the seventh day after feeding they had laid altogether 258 eggs; an average of 43 eggs per female. They refused to feed again, and died within the next 3 days. Another fed female, which was kept under a separate glass chimney, laid 54 eggs. It also refused to feed, and died on the following day. In both dishes the eggs were scattered singly or in small groups on the soil, under the flat stone, as shown in Fig. 1 A.

The variation of the number of eggs in the batches of the same species of *Culicoides* depends not only on the quantity of food taken before oviposition, but also on anatomical differences in their ovaries, in which the number of ovarioles varies considerably in individuals of the same species. Thus, dissection of the five females of *C. vexans* showed the following number of ovarioles in each ovary: 28, 35, 51, 74 and 115 respectively.

THE EGGS AND THEIR HATCHING

The eggs of *C. vexans* are cigar-shaped, and narrower towards the posterior end. They are usually evenly curved, and therefore look crescent-shaped when examined from the lateral aspect, but a small number of them was found curved either at one or both ends (Fig. 1 B). Their length varies between 354 and 378 μ, while the diameter of all of them was the same, measuring 64 μ. They look dark—almost black—when examined under a single lens, but when just laid they are of a light-grey colour. A few of these light-coloured eggs were found in the batches, and they all became black in about 1 hr. Under higher magnification the eggs look dirty-brown. This colour is produced by the combination of the colour of their contents and that of the brown chorion. The surface of the chorion bears very delicate, 2·5 μ long, funnel-shaped processes (Fig. 1C) resembling those of *C. impunctatus*, in which species they were described by Hill. When these processes are examined in the vertical position, they look like very tiny dots or rings. In the anterior and the posterior parts of the eggs the processes are arranged in longitudinal rows, but in the middle part their rows coalesce with each other and are curved. In some parts of the ventral and lateral surfaces they

are arranged in double rows. If the eggs are examined under reflected light and low magnification then the rows of processes look like darkly pigmented ridges.

to those which are present during the winter in their breeding sites. Then the dish was taken to the garden, where it was kept on the ground under cover, which provided protection only against rain.

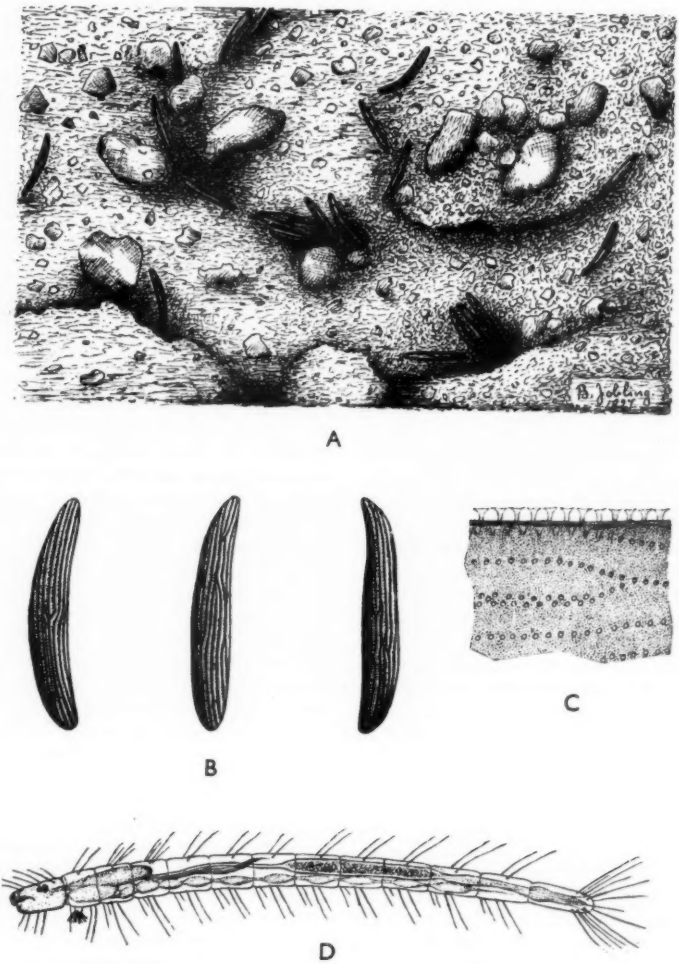


Fig. 1. *C. vexans*. A, eggs *in situ* on surface of soil, × 32. B, three different forms of eggs, × 100. C, chorion, showing funnel-shaped processes, × 1200. D, first-stage larva, × 100.

When eggs were found, one-third of the soil was removed from the dish. Then one side of the remaining soil was arranged in the form of a slope in such a way that it gradually descended to the bottom of the dish, which was covered with $\frac{1}{8}$ in. of water and contained only a few particles of soil. This arrangement of the soil was made to provide the future larvae with conditions more or less similar

In this dish the eggs started to hatch at + 6° C., on 26 October, that is 132 days after the oviposition. During the following 4 days, when the temperature was on an average about the same, many more larvae appeared from the eggs. On 1 November the temperature dropped to + 4·5° C., and only a very few larvae succeeded in escaping from the egg-shells. At this temperature the larvae were almost

stationary, and responded very feebly to the touch. No hatching was observed at + 4° C. on the following day, though the chorion of many eggs was ruptured 2 days before. In this condition the larvae remained until the end of December when the culture was brought to the laboratory, where at room temperature of + 16° C. the larvae escaped from the ruptured eggs in a few minutes, while all the unruptured eggs hatched out in about 3 hr.

Further observations on this culture were then made in the laboratory, at temperatures between 15 and 18·5° C. Immediately after hatching from the eggs, the larvae, after remaining stationary for about 2 min., started to move about and soon disappeared in the soil, where they lived between the wet lower part and the drier upper part. They only occasionally appeared on the surface of the soil or in the water. Their movement on the surface of the soil resembled that of a snake, while in the water they swam by very fast wriggling movements of their bodies, which produced an impression of an elongated figure 8.

The first pupa was found on 1 February and the first adult, male, emerged 4 days later. During the following few days many more males appeared in the culture, and they were gradually succeeded by the females, of which the last specimen emerged on 4 March.

These observations show that in their natural environment the egg stage lasts more than 4 months, and that the minimum temperature at which the larvae can escape from the egg-shell and possibly develop, is about + 5° C. They also show that in this species the larval stage lasts about 6 months. As the temperature of the winter months is very low, the species probably passes this part of the year as the first-stage larva, and completes its development in March and April.

THE STRUCTURE OF THE FIRST-STAGE LARVA

The following description of the larva was made from specimens soaked in slightly warmed caustic potash, and then stained in diluted carbol fuchsin. It has been found that the sensilla and the setae of the head of the first-stage larva of *C. vexans* are homologous to those of *C. nubiculosus*. Therefore, in the present paper they have the terminology which was adopted by Lawson (1951) from that of Saunders (1924).

Immediately after hatching from the egg, the length of the larva is on an average 880 μ, but before the larva begins to move about, it increases to 960 μ. This elongation of the body was also observed by Williams (1951) in *C. tristriatulus* Hoffman. The body of the larva is more or less spindle-shaped, semi-transparent, and consists of three thoracic, and nine abdominal segments (Fig. 1 D).

The head (Fig. 2 A, B)

The head is of light brown colour, cordiform, 80 μ long and 65–66 μ wide in its broadest part. About two-thirds of its dorsal surface is formed by the fronto-clypeus (*fcl.*), which is separated from the rest of the head capsule by the ecdysial or the epicranial suture (*es.*). This compound sclerite was regarded before as the clypeus, but Lawson (1951) indicated that its posterior part belongs to the frons. The anterior part of the fronto-clypeus is convex, and looks broadly rounded when examined from the dorsal aspect. Its lateral margins are curved, while the posterior part is broadly triangular, and bears the darkly pigmented hatching spine (*h.s.*). The chaetotaxy of the fronto-clypeus is the same as that of the third-stage larva of *C. nubiculosus*. Its anterior part has the sensilla '*z*' behind which arise the two setae '*t*' and near the margins of the middle part it bears the setae '*q*'.

Behind the fronto-clypeus lie the two lance-shaped parts (*ver.*) which have each the two pits '*j*'. The position of these parts, in relation to the other regions of the head, indicates that they belong to the vertex. This is also supported by the fact that the postero-dorsal dilators of the pharynx (= posterior pharyngeal muscles of Lawson), which in other insects originate on the vertex, originate on these parts. These parts of the vertex are separated from each other by a very short membranous gap, which represents the greatly reduced coronal branch of the epicranial suture. From the lateral parts of the head they are separated by the angularly curved ridge (*oc.s.*). This ridge is regarded here as the occipital suture, of which the middle part has fused with the dorsal margins of the foramen magnum, while the lateral parts have been bent backwards by the elongation of the postero-ventral and the postero-lateral parts of the head. Owing to these modifications, the dorsal part of the occiput was obliterated, and its lateral parts (*oc.*) separated from the lateral walls of the head by the occipital suture.

The lateral walls of the head are limited above by the epicranial suture and below by the cervical membrane (*m.*). Each of them is composed of the two regions: the gena (*g.*) and the postgena (*pg.*). Owing to the peculiar shape of the head of this larva, and the very small eyes, it is very difficult to define the limit of these two regions. However, the comparison of the head with that of other insects seems to indicate that the part of this wall which is visible from above and extends posteriorly to the eye, must be regarded as the gena, and the rest as the postgena. In the head of this larva the ventral margins of the postgenae, as those of the orthopteroid insects, end at the posterior tentorial pits (*p.t.p.*). Therefore, the part

of the ventral wall which lies between these margins must be regarded as homologous to the cervical membrane. In the larvae of Nematocera the middle part of this membrane has extended anteriorly under the labium as a fold, and has become modified into the sclerotized and toothed plate. In the larvae of Culicidae this plate is usually called the mentum. In *Chironomus* sp. Cook (1944*b*) regarded it as the submentum. Snodgrass (1935) and Anthon (1943) described it as the hypostoma. Lawson used the same terminology as the last two mentioned authors, and regarded it as the secondary outgrowth of the head capsule. The hypostoma (*hp.*) of the first-stage larva of *Culicoides vexans* extends posteriorly to the foramen magnum (Fig. 2 B). It is separated from the postgenae by the cervical membrane (*m.*), and therefore does not form with them the hypostomal suture. Its anterior part is strongly sclerotized and darkly pigmented. The anterior margin of this part is broadly angular and bears two small finger-shaped processes at the apex. Together with the lateral curved margins it forms the sharp corners from which the edges of the curved-inwards cervical membrane extends to the postgenae as darkly pigmented lines.

The chaetotaxy of these parts is as follows: each gena bears in its posterior part the seta '*s*'. The postero-dorsal part of each postgena has the sensillum '*r*' and the two setae '*p*', and on the ventral surface of the head it has the sensillum '*n*' in the anterior part, and the two setae '*w*' and '*v*' near the lateral margin. In each anterior part of the cervical membrane there are present close together the two setae '*o*' while each lateral margin of the hypostoma bears the seta '*y*'. The sensilla '*k*' and '*m*' and the setae '*u*', which were described by Lawson in *C. nubiculosus*, are absent.

The foramen magnum is very large in comparison with the size of the head. It is ringed by the sclerotized posterior margin of the head, which consists of the two dorso-lateral and one ventral parts. The dorso-lateral parts are probably formed by the fusion of the postocciput with the postoccipital suture, while the ventral part, which extends between the posterior tentorial pits (Fig. 2 B), represents the thickened margin of the hypostoma, and is regarded here as the hypostomal bridge (*hp.b.*). On the dorsal surface of the head the dorso-lateral parts are separated from each other by the greatly shortened coronal branch of the epicranial suture, and owing to their fusion with the ridge of the occipital suture (*oc.s.*), the margin of the foramen magnum behind each part of the vertex has a double thickness. The postoccipital suture (*poc.s.*) is distinct only on the ventral wall of the head, where it ends in the posterior tentorial pits (*p.t.p.*), as that of the other insects.

It is seen from the above description that in the first-stage larva the ventral wall of the head is formed by the postgenae and the cervical membrane, of which the middle part is modified into the hypostoma.

According to Lawson, in the fourth-stage larva of *C. nubiculosus* the ventral wall has a different structure, and in such species as *C. delta* and *C. halophilus* it has a short median suture as that of some other Nematocera. Lawson discussed the morphology of this wall at some length, but it seems that it still remains somewhat obscure. Although the head of the fourth-stage larva has not been examined by me, I should like to mention here that in some Nematocera the structure of the ventral wall remains almost the same during the development of the larvae, whereas in the other it undergoes very considerable changes. The larvae of *Culicoides* belong to the second category, and therefore, in order to understand the morphology of this wall in the fourth-stage larva, it should be compared with that of the first stage, because in the latter it shows a more primitive condition than in the former.

The eyes (Fig. 1 D) are situated below the middle part of the epicranial suture half way between the setae '*s*' and the sensilla '*r*'. Each eye is composed of two black spots, which are formed of many granules of pigment (Fig. 2 C). The anterior spot is small and is almost rounded, while the posterior spot is much larger, rotundo-triangular, and looks more or less reniform when examined under a low magnification. The cuticle over the eyes is not unpigmented and is devoid of any special structure.

The antennae (Fig. 2 A, *an.*) resemble those of the fourth-stage larva of *C. nubiculosus*. Each is situated lateral to the labrum, in the depression near the sinuate anterior margin of the gena. In this larva the basal part of the antenna is indistinct, and what is regarded as the second segment, bears three very minute papillae and the seta-like sensillum. On the ventral side, and slightly mesal to this sensillum, there is present a flat, rounded process, which looks more or less triangular when the head is examined from the dorsal aspect.

The labrum (Fig. 2 A, *lm.*, E) is a very broad membranous lobe of the head which slightly projects in front of the fronto-clypeus. Its dorsal wall arises from the anterior margin of the fronto-clypeus and bends antero-ventrally, while the ventral wall merges into the dorsal wall of the cibarium (Fig. 2 G, *cb.*). The anterior margin of the labrum is transverse in the middle and bears two seta-like sensilla. Sensory organs are also present on the ventral wall. They consist of a group of three very small sensilla in each antero-lateral part, near the margin; two larger sensilla in the middle of the anterior part; and two very thin setae in the middle of the posterior part. Besides, this wall bears three

strongly sclerotized hooks (*mes.*) in each postero-lateral part, and in the middle between them a very small plate which has a curved row of five very minute denticles in its posterior part. Each set of the hooks is attached to the rod, which as far as it is possible to see, lies in the membranous wall of the labrum. The anterior narrow part of each rod is strongly bent dorso-laterally, but its attachment to the head capsule I could not make out in my preparations. These rods, together with their hooks, were called the 'premandibles' by Goetghebuer (1925), but as they represent the sclerotized parts of the labrum, the term messores (singular messor), introduced by Cook (1944*a*), is adopted here. It is more probable that they are homologous to the lateral parts of the labrum of the imago, which were described by me in 1928.

The mandibles (Fig. 2 D) are hook-shaped, 25·5 μ long and are very strongly sclerotized. Owing to their dark pigmentation they are much more conspicuous than the other mouthparts. Their appearance, however, changes considerably according to the position of the head under the microscope. This is shown in the two drawings, of which the first represents the left mandible as it usually appears inside the head, when the latter lies in its normal position, while the second drawing shows the same mandible when it is mounted flat on its dorsal side. The outer side of the mandible has a very thick margin. In the basal part this margin is produced into a short process, by which the mandible articulates with the socket of the thickened anterior margin of the head. On the ventral side the basal part is strengthened by the rod-like thickening which extends diagonally from the articulating process to the anterior margin. In the corner formed by this thickening and the margin, the basal part has a very small sensillum. The distal part of the mandible is claw-shaped and ends in a very sharp point. At the base of this part lies the strong conical tooth, and in the basal part of the latter there is a tooth-like process which serves for the insertion of the adductor muscle. In the resting larva the mandibles lie in the preoral cavity, in a slightly tilted position. When protruded from this cavity they move in the vertical plane parallel to each other. They are used for scratching food particles, and probably also as organs of locomotion.

The maxillae (Fig. 2 B, *mx.*) are almost entirely membranous, and therefore are not very distinct. As far as it is possible to see without dissection, each is composed of three segments. Of its first segment only the anterior part is visible outside the preoral cavity. It is rounded, bears four or five very minute sensilla and lies antero-dorsal to the anterior margin of the hypostoma. The second segment is rather broad, with rounded apex, which has five very small sensilla in the antero-lateral part. Dorsally and slightly mesally to these sensilla it bears the third segment, which is the palpus (*pa.*). The latter is short, cylindrical, with four finger-shaped sensory processes arising from its flat rounded apex.

The labium (Fig. 2 F, G, *lb.*). The form of this mouth part is also not clear when the head is examined dorsally or ventrally. In the longitudinal section it looks triangular. Its dorsal surface is sclerotized in the middle, as shown in F, and merges posteriorly into the ventral wall of the common salivary duct (*c.sd.*). Its ventral wall is membranous and is attached to the dorsal wall of the hypostoma, with which it forms a shallow pocket.

The hypopharynx (Fig. 2 F, G, *hy.*). It is shown in the description of the next part of the head that the hypopharynx forms the ventral wall of the cibarial pump. Its most anterior part is almost membranous, and projects over the orifice of the common salivary duct as a very short, broadly rounded flap.

The cibarial pump (Fig. 2 F, G). The cibarium or the cibarial pump of the Ceratopogonidae is a very distinct structure which lies in the middle of the head. It consists of the anterior conical part which acts as a pumping organ, and the postero-dorsal part (*m.cb.*) which breaks the particles of food and moves them into the pharynx. It was called by Goetghebuer (1914, 1919) the 'hypopharynx'. The same term was used by Keilin (1921), who described its several parts in *Tetraphora versicolor* Winn. (= *Dasyhelea obscura* Winn.). The first detailed description of the cibarium was given by Saunders (1924), in *Forcipomyia bipunctata* (F.). He recognized its composite structure but 'for the sake of convenience' also called it the hypopharynx. Mayer (1934) named the whole structure the 'pharyngeal skeleton' (Pharyngealskelett). The ventral wall of the anterior part he called the 'chewing plate' (Kauplatte) and the postero-dorsal part as the 'pharyngealangulus'. Lawson used the term hypopharynx only for the ventral wall of the anterior part. The middle element of the postero-dorsal part he called the 'epipharynx', and homologized it with that of other insects, while the whole structure he named the 'pharynges'.

The term cibarium was introduced by Snodgrass (1935), who has shown that in the adult and in the larvae of Diptera it is formed of the same parts as that of the Orthoptera, and in his other paper (1944) more fully described its structure in the biting and sucking insects. According to Snodgrass the cibarial pump is composed of the following parts: its ventral wall is formed by the extended basal part of the hypopharynx, and is supported on each side by the suspensorial sclerites of the latter, of which the distal rod-like parts he called the oral arms. The dorsal wall of the pump he regarded as the epipharyngeal wall of the clypeus.

A comparison of the anterior part of the cibarial

pump of the first-stage larva of *Culicoides vexans* with the cibarium of the orthopteroid insects shows that it is formed of the same parts as that of the latter. It resembles the cibarial pump of the imago, and resembles a trough-shaped basin. In its posterior part it narrows abruptly to form a median plate which bears on the posterior transverse margin a row of very minute teeth (Fig. 2 F). Its dorsal or

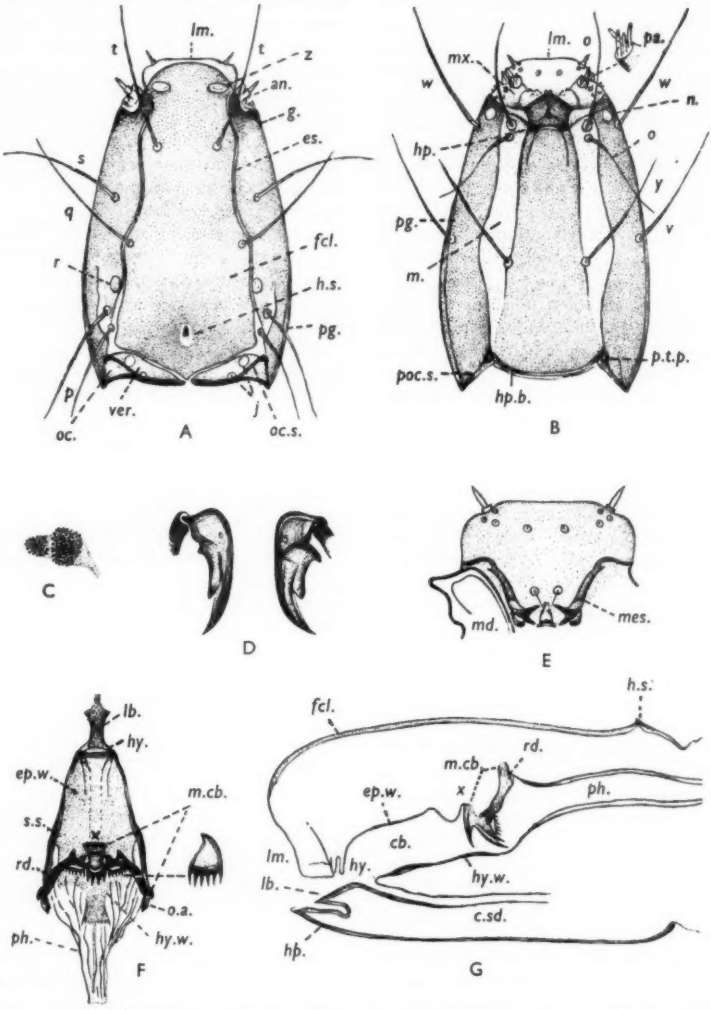


Fig. 2. *C. vexans*. A, dorsal and B, ventral view of head, eyes not shown owing to their destruction by soaking in potash, × 600. C, eye, × 750. D, left mandible, × 800. E, ventral view of labrum showing position of messores (*mes.*), × 1000. F, dorsal view of cibarial pump, mola cibarialis (*m.cb.*) and pharynx (*ph.*), × 750. G, median longitudinal section through head, showing position of hypostoma (*hp.*), labium (*lb.*), hypopharynx (*hy.*), cibarial pump (*cb.*), mola cibarialis (*m.cb.*) and pharynx (*ph.*), × 750.

especially that of the male, in which it was incorrectly described by me (1928) as the pharynx. In the first-stage larva its ventral or *hypopharyngeal wall* (*hy.w.*) is also formed by the extension of the basal part of the *hypopharynx* (*hy.*). It is sclerotized *epipharyngeal wall* (*ep.w.*) is membranous and convex towards the ventral wall, so that the lumen of the cibarial pump looks crescent-shaped in transverse sections. On this wall are inserted the dilator muscles of the cibarium which take their origin on

the clypeus. Both walls are attached to the strongly sclerotized rods (*s.s.*) which represent the *suspensorial sclerites of the hypopharynx*. The posterior parts of these sclerites are the oral arms or the cornua (*o.a.*), on which in the imago are inserted the retractor muscles of the cibarial pump.

The postero-dorsal part of the cibarial pump (*m.cb.*) represents the sclerotization of the posterior part of the epipharyngeal wall of the clypeus. Although this part has been derived from the epipharyngeal wall, as the epipharynx of the other insects, it must not be regarded as homologous to the latter. The epipharynx of the other insects lies under the labrum, and represents a membranous or sclerotized fold of the anterior part of the epipharyngeal wall which lies anteriorly or dorsally, according to the position of the cibarium, to the insertion of the dilator muscles, whereas this part has been derived from the posterior or dorsal part of that wall, which lies posterior to the insertion of those muscles. Therefore, in order to avoid confusion of this part of the cibarium with the epipharynx, I propose to call it the *mola cibarialis*; the term which indicates its relation to the cibarium and also its function.

The mola cibarialis of the first-stage larva consists of the two sclerotized rods (*rd.*), or the 'wing sclerites' as they were called by Keilin (1921) and Saunders (1924). The posterior ends of these rods have the concave ventral surface by which they articulate with the oral arms (*o.a.*) of the suspensorial sclerites of the hypopharynx. From these points each rod curves antero-ventrally and forks at its other end. The anterior branch of the fork forms a broadly triangular process, while the posterior branch is directed towards that of the other rod, and is connected with it by the sclerotized part which resembles an inverted U. The latter is attached to the middle part, which projects inside the lumen of the cibarial pump and bears an anterior and a posterior set of teeth. The anterior set, which is indicated in the drawings F and G by *x*, consists of the three teeth. They project almost vertically into the lumen and look like a small transverse plate when examined from above (Fig. 2 F, ×). The posterior set consists of the four teeth, of which the middle two are longer than the other and almost touching the concave part of the hypopharyngeal wall (Fig. 2 G, *hy.w.*). To each side of the middle part, under the posterior branch of the fork, is attached the triangular comb-like plate (shown separately in Fig. 2 F) which bears on its posterior margin six small teeth. The whole structure is suspended like a pendulum from the oral arms, and during the feeding of the larva moves to and fro inside the cibarium breaking and grinding the particles of food and moving them into the pharynx.

The mola cibarialis is present only in the larvae of the Ceratopogonidae, where it performs the function of the mouthparts such as the mandibles and the maxillae, the structure and the position of which in this family are not adapted for the mastication of food. Its structure shows well-marked generic differences, which are probably correlated with the nature of food of the larvae. For instance, in the larvae of *Culicoides*, which feed on coarse particles of decomposing vegetable matter, Diatoms and other Algae, the middle part of the mola and its comb-like sclerites are armed with teeth, but in *Tetraphora* and *Forcipomyia*, which feed on moulds and other fungi, these parts bear curtain-like membranes, which are strengthened by numerous very thin parallel rods or ridges. In the larvae of these two genera the grinding of the food is done by the rough ventral surface of the middle part of the mola, which is convex and has either transverse ridges or denticle-like protuberances.

The pharynx (*ph.*) is membranous and is more or less funnel-shaped. The wall of its anterior part is attached to the posterior margin of the rods of the mola and to that of the hypopharyngeal wall of the cibarium. It has the same muscles as that of the imago, which consist of the antero-dorsal, postero-dorsal and the postero-lateral dilators.

The thorax (Fig. 3 A, B)

The thorax is attached to the head by a short membranous neck (*n.*). In the posterior part of the ventral wall of the neck and close to the margin of the first thoracic segment lies the prothoracic pseudopod (*p.p.*). It consists of the broadly convex protuberance, which is slightly depressed along the middle and looks bilobed when viewed laterally. The surface of the pseudopod is sparsely covered with microscopic spines, and on each side of the depressed middle part it bears four long thin hooks.

The segments of the thorax are barrel-shaped when viewed from above. The prothoracic segment is slightly smaller than the metathoracic segment, while the mesothoracic is the largest segment of the body. These segments bear various setae, and those which are described here, are indicated by the same letters which were adopted by Lawson in his description of the larva of *C. nubiculosus*. The dorsal surface of the prothoracic segment bears the two setae '*a*' and two small setae '*b*' and '*c*' near each lateral margin. On its ventral surface this segment bears the following setae: two long setae '*i*' near the anterior margin; one long seta '*f*' near each lateral margin; small seta '*h*' between the long setae; and a group of four closely situated setae '*g*' in each postero-lateral part. The last-mentioned setae are homologous to those of *Tetraphora versicolor*, in which species it was shown by Keilin (1921) that they represent the vestigial sensory organs of the legs. On this segment the setae '*d*' and '*e*' are absent.

In the other thoracic segments I was able to see clearly only the setae '*a*', '*b*', '*c*' and '*d*' of the dorsal surface, whereas the other setae were indistinct owing to the crinkles in the integument formed in the course of preparation.

The abdomen (Figs. 1 D and 3 C)

The abdominal segments, except the ninth, are barrel-shaped. Towards the posterior end of the body they become slightly narrower and longer. In the middle of their dorsal and ventral surfaces they bear two long setae and in the other parts several very much smaller setae. But the structure and position of the latter were not clear owing to their microscopic size and the crinkles in the cuticle. The ninth or the anal segment is slightly conical, compressed in the posterior part which bears seven long setae on its lateral surfaces. The anus is a slit-like aperture, which lies between the flat, valve-shaped posterior parts of this segment.

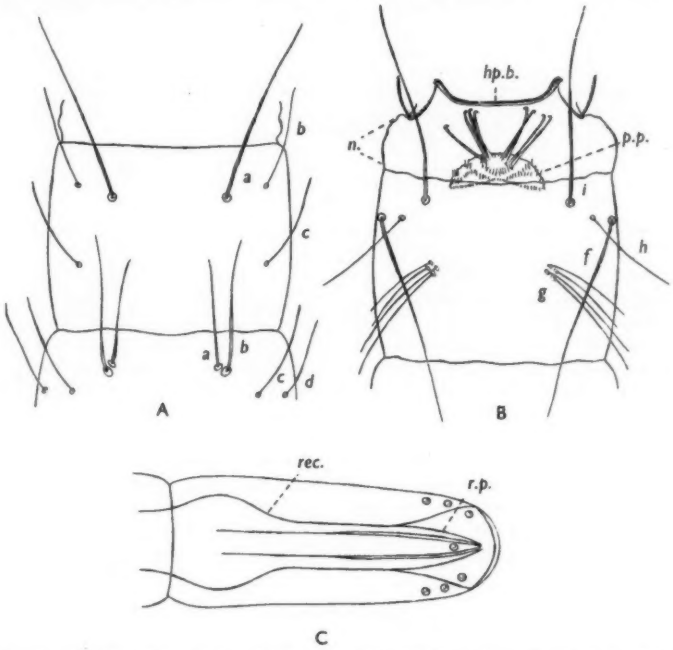


Fig. 3. *C. vexans*. A, dorsal view of the first and part of the second thoracic segment. B, ventral view of neck (*n.*), prothoracic pseudopod (*p.p.*) and first thoracic segment. C, ninth abdominal segment, showing position of rectal papillae (*r.p.*) in posterior part of rectum (*rec.*), × 600.

The internal anatomy

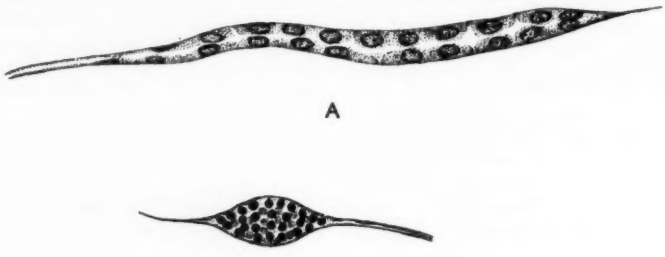
The alimentary canal (Fig. 1 D). In the posterior part of the head the pharynx is followed by the very thin, long oesophagus, which extends posteriorly into the posterior part of the third abdominal segment, where it ends in the cardiac part of the mid-gut. The mid-gut or the ventriculus is more or less sacciform, about 22 μ in diameter. It is more distinct than any other part of the alimentary canal, owing to the presence in its lumen of the food material. In all the larvae which were examined, the contents of the mid-gut consisted of numerous greenish fragments of algae, small particles of detritus and a few common Diatoms of the soil, *Novicula mutica* Kütz. The mid-gut extends into the anterior part of the seventh segment, where it is followed by the hind-gut. At the point of junction of the mid-gut with the hind-gut there are attached the two Malphighian tubes. In this larva only the proximal parts of the tubes are visible, whereas the rest of them is obscured by the abdominal muscles and the fat body. The hind-gut consists of two parts: the ileum and the rectum. The ileum lies inside the seventh and the eighth abdominal segments. It is quite distinct owing to the presence in its lumen of the excretory product which is very light brown. The rectum occupies the ninth segment. It is more or less pear-shaped in its anterior part and has the four lance-shaped rectal papillae (Fig. 3 C, *r.p.*). These papillae usually lie inside the rectum, but from time

to time the larva protrudes them through the anal opening and holds them outside the ninth abdominal segment.

The salivary glands (Fig. 4 A) lie inside the first three abdominal segments, lateral to the oesophagus. Each gland is spindle-shaped, 85μ long, and is composed of nineteen or twenty comparatively large cells.

united with each other by short connectives and lie in the middle of the ventral part of their respective segments. The last pair is formed by the fusion of the ganglia of the seventh and eighth abdominal segments and is much larger than the other pairs.

The respiratory system and the dorsal vessel are indistinct in the unstained first-stage larva.



A

B

Fig. 4. *C. vexans*. A, salivary gland. B, gonad (haematoxylin). × 900.

The nervous system (Fig. 1 D). The brain and the suboesophageal ganglion are not distinct in the unstained larva. They are obscured by the large imaginal buds of the head and legs, which occupy the largest part of the thorax. The three pairs of the thoracic ganglia are displaced posteriorly by the imaginal buds of the legs, so that the prothoracic ganglia lie behind these buds in the posterior part of the mesothorax. The mesothoracic ganglia lie in the metathorax and the metathoracic ganglia lie partly in the metathorax and partly in the first abdominal segment. The abdominal nervous system consists of the seven pairs of ganglia. They are

The gonads (Fig. 4 B) are broadly spindle-shaped, 17·5μ long, and lie in the sixth abdominal segment, dorso-lateral to the posterior part of the mid-gut. Each of them is composed of many cells, which are enclosed in the connective tissue coat or peritoneal sheath. In the anterior part of the gonad the peritoneal sheath forms a very thin terminal filament, and in the posterior part of the gonad it extends postero-ventrally as a very thin tube, enclosing the strand of very small epithelial cells. During the subsequent development this part of the gonad becomes either a vas deferens in the male or a lateral oviduct in the female.

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EXPLANATION OF REFERENCE LETTERING

an. antenna; *cb.* cibarial pump; *c.sd.* common salivary duct; *es.* epicranial suture; *ep.w.* epipharyngeal wall of cibarial pump; *fcl.* fronto-clypeus; *g.* gena; *hp.* hypostoma; *hp.b.* hypostomal bridge; *h.s.* hatching spine; *hy.* hypopharynx; *hy.w.* hypopharyngeal wall of cibarial pump; *lb.* labium; *lm.* labrum; *m.* cervical membrane; *m.cb.* mola cibarialis; *md.* mandible; *mes.* messor of labrum; *mx.* maxilla; *n.* neck; *o.a.* oral arm; *oc.* occiput; *oc.s.* occipital suture; *pa.* palpus; *pg.* postgena; *ph.* pharynx; *poc.s.* postoccipital suture; *p.p.* prothoracic pseudopod; *p.t.p.* posterior tentorial pit; *rd.* rod of mola cibarialis; *rec.* rectum; *r.p.* rectal papillae; *s.s.* suspensorial sclerite of hypopharynx; *ver.* vertex.

ON A SPOROZOON IN THE COELOMIC CORPUSCLES OF *PHASCOLOSOMA MINUTUM* KEFERSTEIN (SIPUNCULOIDEA)

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(With 19 Figures in the Text)

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I. INTRODUCTION

The coelomic cavity of the sipunculid worm *Phascolosoma minutum* Keferstein (= *Petalostoma minutum* Keferstein) contains a fluid in which large numbers of cells are present. These were roughly classed by Cuénot (1900) as haematids (containing haemerythrin) and amoebocytes, a terminology that it is convenient to adopt here. Ikeda (1914) makes a reference to 'cysts of an unknown Sporozoan (?)' in the coelomic corpuscles of this worm. Dr H. N. Ray and Prof. D. L. Mackinnon, working at the Marine Biological Station at Plymouth in 1931, observed cysts and other stages of a sporozoan in both haematids and amoebocytes. They thought that these might all be referable to the life history of some aberrant telosporidian, possibly related to *Selysina perforans* recorded by Duboscq (1918) from the blood cells of an ascidian.*

I was privileged in 1947 to examine the slides prepared by Dr Ray, when Prof. Mackinnon suggested that I should try to work out the life history of the parasite. I had hoped to supplement his material with more of my own preparations; I did not anticipate that this would present any difficulty, as the infection in Dr Ray's material was very heavy. Unfortunately, I found that the infection in 1947 was much lighter than that of 1931, and during the period 1947–51 it became progressively lighter. Very few infections comparable with those seen by Dr Ray have been observed. (This is due, I think, at least in part to overfishing of the collecting grounds; in 1950–1 the *Phascolosoma* obtained were, in general, young specimens.) As a result, I have not been able to study the parasite in the living condition as much as I could have wished. Consequently, I have also been unable to establish the complete life history of the parasite; and the notes here recorded are incomplete and inconclusive. But I feel that it is worth putting on record the present findings, and discussing, tentatively, the possible affinities of the parasite, in the hope that, should a rich infection reappear in the future, it may then prove possible for some protozoologist to confirm or correct these suggestions.

* In course of personal correspondence, Dr H. Pixell-Goodrich many years ago informed Prof. Mackinnon that she also found this same parasite in *Phascolosoma minutum* when working at Plymouth in 1934, and studied it at intervals from 1934 onwards. She was inclined to regard it as a haplosporidian. In later seasons she also noted the marked decline in the numbers and richness of the infection, to which I refer above. On learning that I was working on this enigmatic organism from the material at King's College, she generously left the field to me. I take this opportunity of acknowledging my indebtedness to her.

II. MATERIAL AND METHODS

To a large extent my observations have been based on the excellent slides of smears of the coelomic fluid, made by Dr Ray and very kindly lent to me by Prof. Mackinnon, from animals collected at

Plymouth. These smears were fixed in Brasil's modification of Bouin-Duboscq fluid, stained in Delafield's or Heidenhain's haematoxylin and counterstained with Chromotrope 2R.

My own material was also obtained from *P. minutum* collected at Rum Bay, Plymouth, and examined either at the Laboratory, Plymouth, or in London. In April, August and October 1947 193 animals were examined; 24 in February 1948; 68 in April 1949; 76 in August 1950; and 60 in April and August 1951. The infection rate for these animals was less than 20%, and most of the infections were light; often only a few examples of a single stage of the parasite were seen from a preparation of one animal. In addition, 28 specimens from Bangor have been examined, through the courtesy of Prof. Rogers Brambell; in these, a single slight infection was observed.

In the earlier part of the work, the entire coelomic fluid of a worm was expressed on a slide and examined, and the rest of the body was then discarded. Later, it was found possible to withdraw only a small quantity of the coelomic fluid, and to keep the animal alive afterwards; by this means several samples could be taken from the same host over a period of 3–4 weeks, and the animal could be fixed and sectioned later. All the samples were first examined in the living condition, in sea water diluted with one-third of its volume of tap water, which caused no shrinkage of the haematids. Infected samples were then fixed by irrigation with Bouin's fluid in sea water, or with Brasil's modification of Bouin-Duboscq fluid. The slides were stained in Delafield's, Ehrlich's or Heidenhain's haematoxylin, and counterstained with Chromotrope 2R.

Longitudinal sections of whole *Phascolosoma* were also examined, but these did not provide any additional information. Attempts were made to infect animals experimentally, to study the time of development of various stages of the parasite, and to release the cyst contents experimentally; these attempts are described on pp. 168–9.

III. DESCRIPTION OF THE PARASITE

The forms of the parasite found in the coelomic fluid may be conveniently described under the headings of *merozoites*, *schizonts* and *cysts*. The possible significance, and the relationship of these stages to one another, are discussed later on pp. 165–8.

(1) *Merozoites and trophozoites*

Since in the available material it is difficult to determine the stage at which a merozoite becomes a trophozoite, the term merozoite is here used to denote the free stages of the parasite, without an envelope of any sort, occurring in the plasma of the coelomic fluid, while the forms found within the haematids are regarded as trophozoites. They are all products of schizogony, but there are certainly two kinds.

(*a*) *Micromerozoites and trophozoites.* These are bodies approximately $8 \times 2{\cdot}5\,\mu$ when fully grown, pointed at one end and bluntly rounded at the other. The nucleus lies close to the pointed end, and its chromatin is compact; the peripheral chromatin appears as a continuous ring, and not as separate granules, and an eccentric karyosome is usually visible. The micromerozoites are free in the plasma of the coelomic fluid (Fig. 1), while the microtrophozoites occur singly in the cytoplasm of haematids (Fig. 2), but occasionally two (rarely more) are present in one haematid.

(*b*) *Macromerozoites and trophozoites.* These have a more elongated body than the micromerozoites, and both ends are blunt. They may be as large as $12 \times 3{\cdot}5\,\mu$, but the majority measure $10 \times 3\,\mu$. The nucleus is more centrally placed than that of the micromerozoites and microtrophozoites, though it is still slightly towards one end; the peripheral chromatin can be seen as separate granules, and the karyosome is always visible. The macromerozoites are occasionally found free in the plasma of the coelomic fluid, but they are never as numerous in this situation as are the micromerozoites (Fig. 3). The macrotrophozoites are common in the cytoplasm of haematids, sometimes occurring singly (Fig. 4), but often in groups of from two to six in one haematid, haphazardly arranged (Fig. 5). Haematids that contain such a group of macrotrophozoites are somewhat hypertrophied.

(2) *Schizonts*

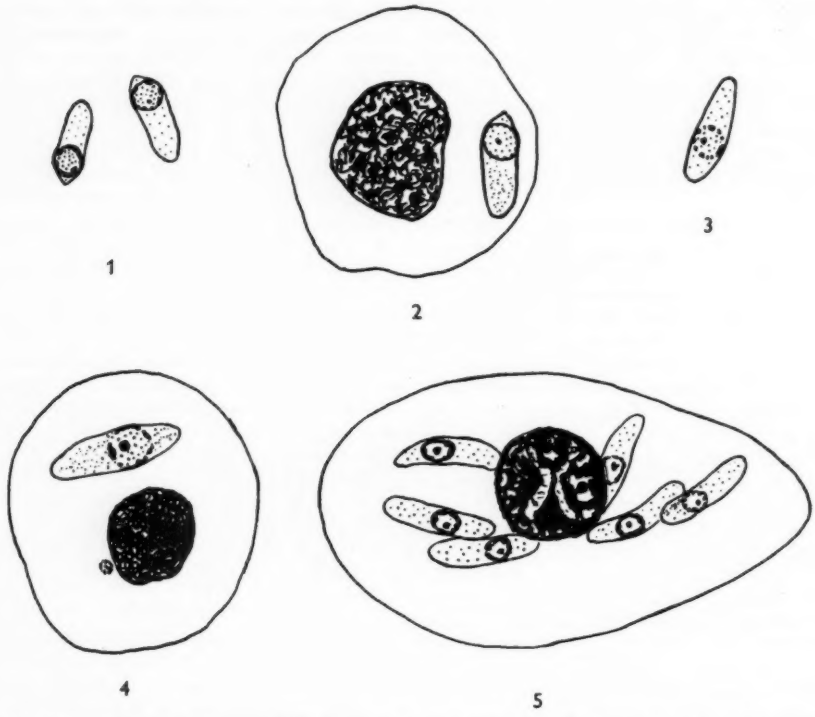
Schizogony takes place within a tough envelope, or 'capsule', in a greatly hypertrophied haematid. This capsule, when mature, may contain a hundred or more micromerozoites, or less than fifty macromerozoites; the microschizonts tend to be larger than the macroschizonts, and may have a thicker envelope, but in other respects the form of the capsule is the same in both types. The mature capsule varies in size from about $20 \times 12\,\mu$ to $50 \times 25\,\mu$. The outline is usually oval or rounded, but it may be of an hour-glass shape, a form which is possibly due to the fusion of two developing schizonts in one haematid. The wall of the capsule is approximately $2\,\mu$ thick, and experiments have shown that it is tough and somewhat elastic; its inner surface is smooth, but the outer surface is raised in blunt sculpturings. Not infrequently the wall projects at one point as a 'spout', presumably for the escape of the contained merozoites. (In one living preparation, merozoites were seen apparently emerging from the spout.) Usually the remains of the haematid surround the envelope, but sometimes these dis-

integrate, leaving the capsule free in the plasma (Figs. 6, 10).

(*a*) *Development of the microschizont* (Figs. 7–9). In my opinion, a micromerozoite free in the plasma enters a haematid, where it becomes a trophozoite. It increases in size, and its nucleus divides rapidly. Though it is not easy to make out the form of the nuclear division, it appears to be a true mitosis, with the separation of a small number of chromosomes. During this phase the wall of the capsule begins to develop; at first it is thin, smooth and transparent, and difficult to see in living material; later it becomes thicker, roughened on the outer surface, and the spout appears. The haematid hypertrophies, at first slowly, and later more rapidly, to keep pace with the growing schizont. It usually remains as a thin membrane closely applied to the wall of the capsule, with its nucleus pushed to one side.

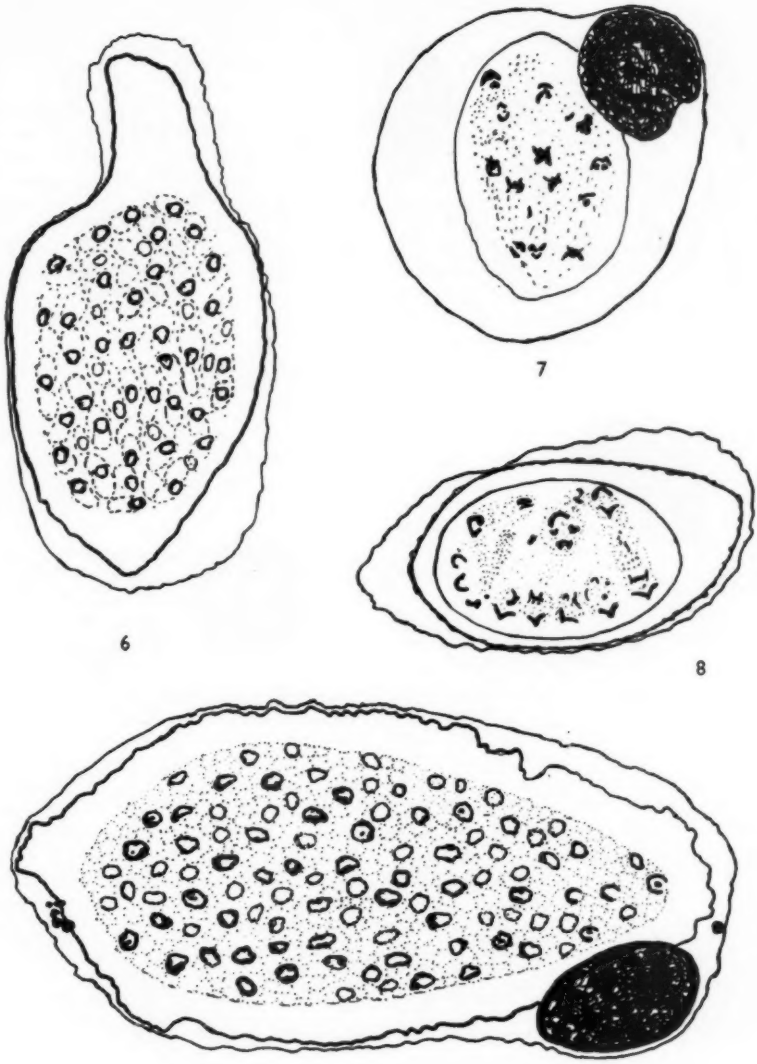
Repeated nuclear divisions in the schizont give rise to a hundred or more nuclei like those of the micromerozoites. Cleavage of the cytoplasm around the nuclei takes place only when the nuclear division is completed, so that only when schizogony is complete can the outlines of the individual merozoites be distinguished. The micromerozoites escape from the capsule when fully formed, probably one at a time, by way of the spout, and invade fresh haematids, usually singly, but sometimes two entering one haematid. It seems likely, from the pattern of infections, that microschizogony is repeated.

(*b*) *Development of the macroschizont* (Figs. 11–14). Eventually the micromerozoites develop the potentiality of macroschizogony. Like those of earlier generations, they penetrate haematids and enlarge to trophozoites, but the early nuclear divisions are



Figs. 1–5. Merozoites and trophozoites of sporozoon in coelomic fluid of *Phascolosoma minutum*. (× 2100.) All drawings were made from fixed and stained preparations, with the aid of a camera lucida.

Fig. 1. Micromerozoites free in the plasma of the coelomic fluid.
Fig. 2. Microtrophozoite within a haematid.
Fig. 3. Macromerozoite free in the plasma of the coelomic fluid.
Fig. 4. Single macrotrophozoite within a haematid.
Fig. 5. Group of six macrotrophozoites in a single hypertrophied haematid.



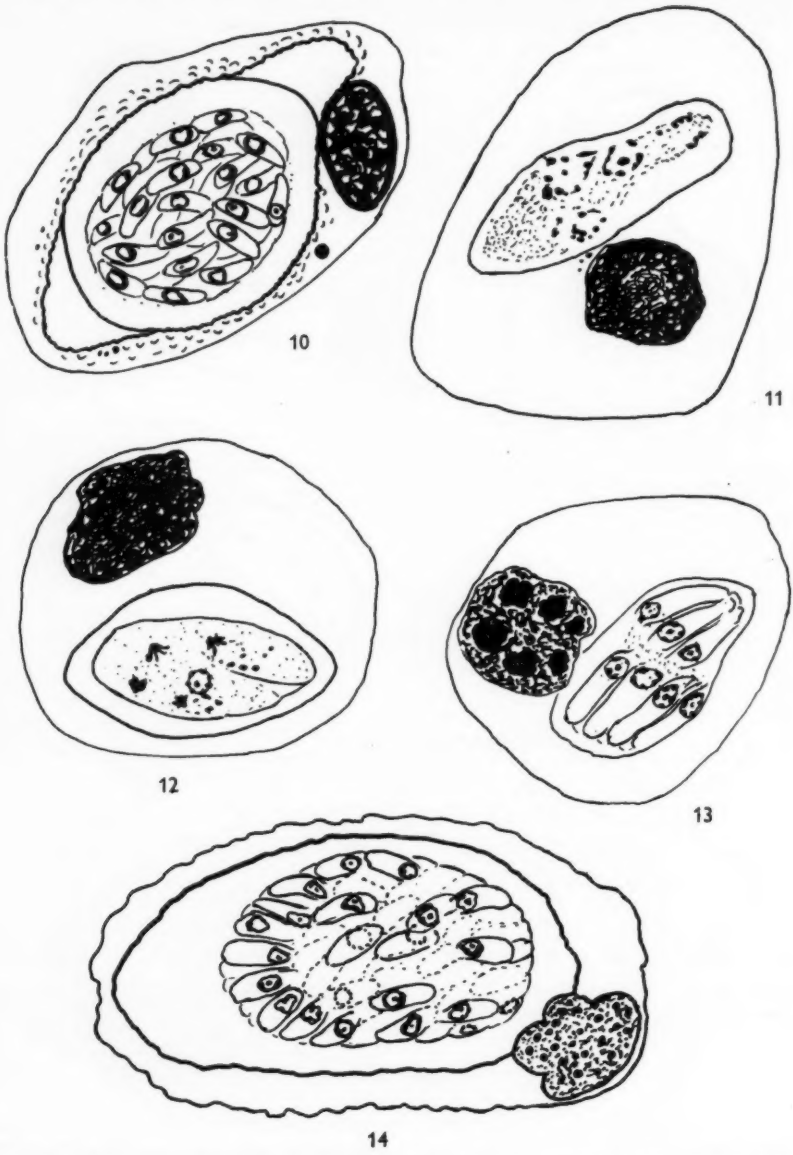
Figs. 6–9. Microschizonts within haematids of the coelomic fluid. (× 2100.) All drawings were made from fixed and stained preparations, with the aid of a camera lucida.

Fig. 6. Mature microschizont and capsule with a 'spout'; nucleus of haematid not visible.

Fig. 7. Young microschizont showing nuclear division; capsule wall little developed.

Fig. 8. Later stage of development of microschizont; capsule wall more pronounced.

Fig. 9. Microschizont almost mature; haematid greatly hypertrophied and closely investing capsule.



Figs. 10–14. Macroschizonts within haematids of the coelomic fluid. (× 2100.) All drawings were made from fixed and stained preparations, with the aid of a camera lucida.

Fig. 10. Mature macroschizont and capsule with approximately fifty merozoites.

Fig. 11. Young macroschizont showing nuclear divisions.

Fig. 12. Later stage of development of macroschizont; capsule wall more pronounced.

Fig. 13. Small capsule in which macromerozoites are almost completely differentiated.

Fig. 14. Macroschizont almost mature; haematid greatly hypertrophied.

less rapid than in the microschizonts, and are accompanied by a partial cytoplasmic cleavage; the chromosomes can be seen more clearly in these divisions (Figs. 11, 12). The growing schizont may be surrounded by a clear vacuole rather than by a definite wall. At the end of macroschizogony the capsule usually contains about thirty to fifty macromerozoites, but occasionally fewer are produced (Fig. 13); their outlines, which become apparent during development, are then very clear.

The macromerozoites are released from the capsule into the plasma of the coelomic fluid, and they thence penetrate fresh haematids, quite frequently several (as many as six) entering a single cell.

(3) *Encysted forms*

What appears to be the final stage of the parasite as it occurs in *P. minutum* is a monozoic spore-like body, found in the globule-laden amoebocytes of the coelomic fluid (Fig. 15). In heavy infections there may be as many as six such bodies in a single amoebocyte. Until I have discussed their significance I shall refer to these forms by the non-committal term of 'cysts'.

The 'cysts' have an elongated oval outline, approximately $24 \times 7\,\mu$, and taper towards the ends, which are prolonged into tufts of from three to six spine-like projections. These spines are normally bunched together and somewhat curved while the 'cyst' is within the amoebocyte, but straighten and diverge from one another when the amoebocyte disintegrates (Fig. 16). The cyst wall is about $1\,\mu$ thick at the sides, although in places it may be rather more; the wall at the ends, near the spines, is considerably thicker. Sometimes one gets the impression that it is formed of a smooth endocyst, with an epicyst from which the spines are developed.

The living contents of the 'cyst' have a gregarinula form, which completely fills the cyst; in fixed preparations the contents tend to shrink away from the wall. The cytoplasm is granular, and occasionally an oblique line of darker-staining granules can be seen. The nucleus, between 2 and $3\,\mu$ in diameter, is set well towards one end; it resembles that of a macromerozoite, the peripheral chromatin appearing as separate granules. There is commonly a vacuole lying just behind the nucleus.

The youngest stage of the cyst within an amoebocyte is surrounded by a thin membrane. During development, the contents grow a little, and especially broaden (Fig. 18), and the wall increases in thickness, the tuft of spines developing last. When an amoebocyte contains several cysts, the spines are abortive. The cysts are released into the plasma of the coelomic fluid by rupture or disintegration of the amoebocytes.

I have not been able to determine with certainty the relationship of the cyst contents to other stages of the parasite, but it seems probable that they develop directly from macromerozoites. It is possible that these may be ingested by amoebocytes while free in the plasma, but it is more likely that infection occurs only when an amoebocyte ingests a haematid containing one or more trophozoites produced by macromerozoites; necrotic remains of haematid nuclei are not uncommon within amoebocytes. Possibly a slight membrane is formed around the trophozoite while it is still within the haematid, which protects it against digestion by the engulfing amoebocyte, for, as noted above, the earliest stage in an amoebocyte already had a thin envelope (see Fig. 17, where an amoebocyte is apparently ingesting such a trophozoite). When the engulfed haematid contains several macrotrophozoites, these all develop into cysts in the same amoebocyte (Fig. 19).

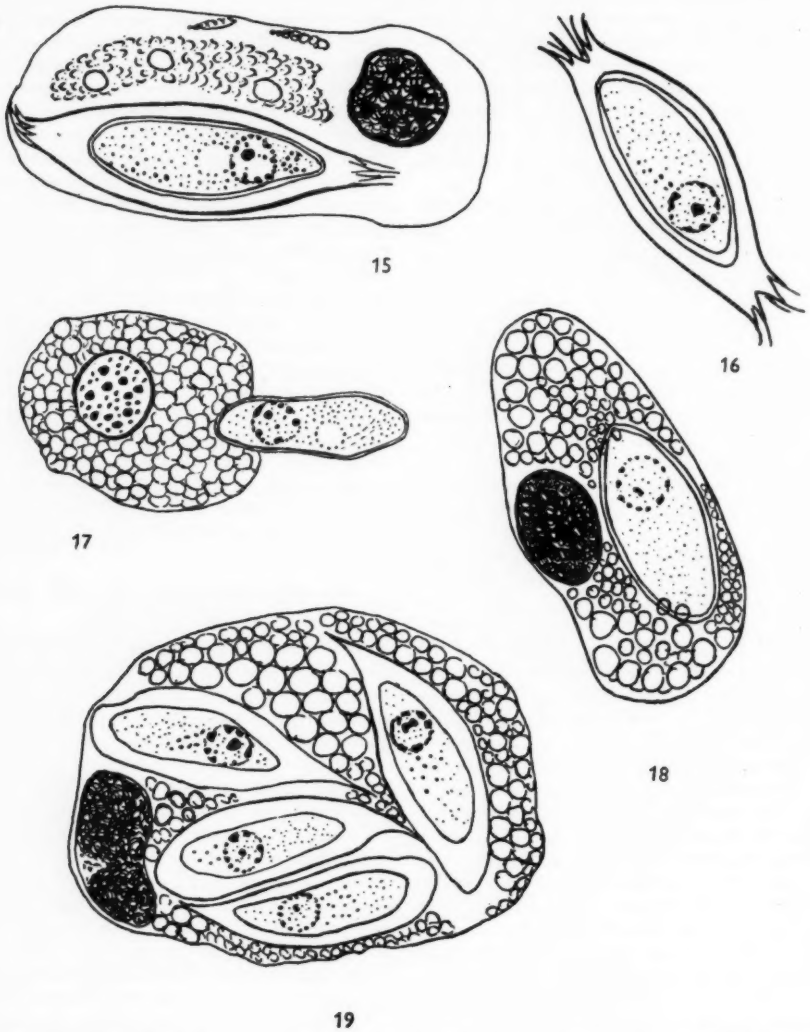
In only one instance have I seen the contents apparently emerging from a cyst, and this, for reasons discussed below, I consider may be an artefact: in this isolated case the emergence appears to be from the side of the cyst, and there is no sign of a micropyle in the wall.

IV. RELATIONSHIP OF THE STAGES OBSERVED

Almost all of these observations have been made from different specimens of the host, and it has not been found possible to establish the relationship between the different forms by continuous observations on living material (see p. 168). The question therefore arises, are all these forms stages of a single parasite, or are two or more different organisms present in one host? The evidence suggests that they are all phases in the life history of one organism. The nuclei of the macromerozoites, macrotrophozoites, macroschizonts and 'cysts' are identical; those of the micromerozoites and microtrophozoites have the same general form, but the chromatin is more compact, a difference which may be due to concentration of the chromatin in the smaller nuclei. The capsules of both types of schizonts are identical, which suggests that the two types of merozoites are in fact both from the same organism. In heavy infections, all the stages described may be present in one sample of coelomic fluid; but in lighter infections there are frequently associations of stages which suggest the relationships described above. For example:

(1) When schizonts are present without cysts, they may be of both types, but most are microschizonts.

(2) Free micromerozoites, or single microtrophozoites in haematids, are found with developing schizonts.



Figs. 15–19. Encysted forms of sporozoon in coelomic fluid of *Phascolosoma minutum*. (× 2100.) All drawings were made from fixed and stained preparations, with the aid of a camera lucida.

Fig. 15. Mature 'cyst' within the cytoplasm of an amoebocyte.

Fig. 16. 'Cyst' free in the plasma of the coelomic fluid, with the spines spread out.

Fig. 17. Amoebocyte ingesting a young 'cyst' or trophozoite.

Fig. 18. Young 'cyst' within amoebocyte, before the cyst wall has fully developed.

Fig. 19. Amoebocyte containing a group of four 'cysts'.

(3) Macroschizonts, macrotrophozoites in haematids, and young cysts are commonly found together in one preparation.

(4) Multiple infections of haematids with macrotrophozoites are commonly associated with multiple infections of amoebocytes with 'cysts', and the rate of these infections is of the same order—up to five or six bodies in a haematid or amoebocyte.

(5) While, rarely, capsules are found in amoebocytes, completely naked trophozoites are never present there, all stages within amoebocytes having an envelope of some kind. 'Cysts' are never found in haematids.

The different stages, then, appear to be referable to the life history of a single organism, and the above account of the relationship between them represents, I think, the most probable course of the life history of the parasite within the host *P. minutum*, and conforms with the patterns of infection. But these stages almost certainly do not constitute the complete life history. It is possible that stages occur in other organs of the host: serial longitudinal sections of fifteen whole *Phascolosoma*, known to have infected coelomic corpuscles, were cut and examined, but no form of the parasite in any other tissue was observed. (Owing to the scarcity of infection in my own material, this line of investigation was not followed as fully as I could wish, while all the preparations made by Dr Ray were from the coelomic fluid only.) The onset of the sexual phase in a sporozoan life history is often relatively brief, and consequently the stages crucial for classification may be unrepresented in a limited material; this is especially true of the Coccidia. I examined some 400 worms at different seasons over a period of 5 years, but found no signs of gamete formation, fertilization, or the development from the zygote that might give the essential clues. So far as I can judge, therefore, no stage but the schizogonic occurs in *P. minutum*.

V. AFFINITIES OF THE PARASITE

It appears, then, that the stages seen in the coelomic corpuscles of *P. minutum* are the schizogonic phases only in the life history of a sporozoon, and result in the formation of encysted bodies. Should the organism be referred to the Haplosporidia or to the Telosporidia? Its habitat, the coelomic fluid of an invertebrate, is a common one for the Haplosporidia, and the monozoic 'cyst' with its terminal tuft of spines does in some degree resemble the spores of this group. But there is no evidence that the 'cysts' are formed within plasmodia, the schizonts are not plasmodial, the merozoites are gregariniform, and no amoebula stage has been seen. It appears more probable that the organism is a telosporidian; the schizogonic stages, intracellular and repeated, and the form of the merozoites, suggest a coccidian affinity. What can be seen of the nuclear divisions in the schizont also supports this view; there is no indication that the nuclear membrane persists, nor does a centriole appear to be present. Can a position within the Coccidia be assigned to the parasite on the evidence available?

Prof. Mackinnon first thought that it might be related to *Selysina perforans*, parasitic in the blood cells of the ascidian *Stolonica socialis* (Duboscq, 1918). Duboscq was uncertain of the affinities of *Selysina*; he placed it in the ill-defined group of 'Selysinides', provisionally classified as 'in the neighbourhood of' the Coccidiomorpha. (Wenyon, 1926, considered that it should probably be grouped with the Aggregatidae.) With the material at his disposal Duboscq could only suggest a possible life history; he thought that a sporozoite, emerging from a monozoic 'spore', gave rise to a schizont, from which schizozoites repeated the cycle. He described two kinds of capsules, one having a thin membrane and another with a thick wall, containing merozoites and 'heliospores'; he believed that those with the thick walls might be gametocysts. The habitat of the parasite of *Phascolosoma* is not unlike that of *Selysina*, which it also resembles in the 'cyst' ('spore' of Duboscq) and encapsulated schizonts; it differs therefrom, however, in the method of production of the merozoites.

Coelomic corpuscles like those of *Phascolosoma* are not common amongst invertebrates; in form and function they are comparable with the blood corpuscles of vertebrates. Prof. Mackinnon has suggested that this parasite of *Phascolosoma* might be distantly related to the Haemogregarinidea (Haemococcidia), with a life history similar to that of *Karyolysus lacertae*, parasitic in the blood corpuscles of *Lacerta* (see Reichenow, 1921). In the Haemogregarinidea schizogony may occur in red blood corpuscles, and the trophozoites are usually enclosed in a membrane of some kind; the gamonts are also usually produced in red blood corpuscles, though leucocytes may also be infected. There is little differentiation in the early stages between the male and female gamonts. In *Karyolysus lacertae* two types of merozoites are formed, both types of schizont being enclosed in a thin capsule. The macromerozoites repeat the schizogonic cycle, while the micromerozoites, invading red blood corpuscles, give rise to male and female gamonts, very similar in form, each enclosed in a stout cyst. These gamonts only develop in a secondary host, a mite.

If my interpretation of the life history of the parasite of *Phascolosoma* is correct, then it conforms in a general way with that of *Karyolysus*. It is true that the capsule wall is much thicker than that depicted for *Karyolysus*, and that the cyst wall is more elaborate than that of the gamonts of that

genus; but this may be associated with the special features of the habitat, as is discussed below. The production of two kinds of merozoite, and the development of a monozoic cyst (gamont) is similar in both parasitic organisms. On this analogy, the stages described here in *Phascolosoma* are the schizogonic stages only, with the formation of the gamonts. The micromerozoites serve to propagate the schizogonic phase, while the macromerozoites give rise to gamonts, which become encysted; they probably require a secondary host to complete their development. In consequence, it is to be expected that no emergence of the 'cyst' contents will occur in the coelom. The 'cysts' may be passed out of the body of the worm via the nephridiopore, and so infect another animal, or the worm may be eaten by a predator.* If not an active predator, the secondary host of the parasite in question must be an animal living in close association with *Phascolosoma*, which lives in a confined environment in the mud in shale rocks. I am inclined to think that the 'cysts' are passed out of the body of the primary host, and are eaten by another microfeeder; the tufts of spines on the cysts are admirably suited for entanglement with mucus. I have examined the guts of specimens of the polychaetes *Polydora* spp. and *Pygospio elegans* which were collected with the *Phascolosoma*, but have found no structure resembling the sporozoan with which I am concerned.

A further point which arises is the function of the very thick wall of the capsule surrounding the schizonts. Most probably it acts as a protection against phagocytosis by amoebocytes in *Phascolosoma*, since haematids are ingested by amoebocytes. Occasionally a capsule is found in an amoebocyte, the haematid presumably having been engulfed but the capsule having resisted digestion. It is possible that capsules, like 'cysts', may pass out of the body of the worm by the nephridiopores, and re-infect another *Phascolosoma* directly, for the host animals are very gregarious. This would involve the penetration of the gut wall by merozoites before invasion of the haematids in the coelomic fluid of the new sipunculid host could take place.

VI. EXPERIMENTAL

It will be apparent from this discussion of the observations that there are three phases where further evidence is desirable: (1) the evidence of relationship between stages is, in some instances, empirical; (2) the form of body after emergence from the 'cyst', which might help to settle the affinities, has not been observed; and (3) the existence of a secondary host has been deduced but not demonstrated. Attempts were therefore made to obtain further information on these points by experimental means. Owing to the sparse infections in my material, it was often necessary to use the coelomic fluid of one animal for several experiments, and to follow the fate of only three or four 'cysts' or capsules in an experiment. This fact limits the value of these tests; it is possible that the methods recorded might have succeeded with larger samples containing many 'cysts' and capsules of different degrees of maturity.

(1) *Development experiments*

In a number of worms with slight infections, the coelomic fluid contained examples of a single stage of the parasite only. This fact appeared to offer a chance to study the development of the particular stage, when it was found possible to keep the host animals alive and healthy for as many as 20 days, and to take several samples of the coelomic fluid during this time. In a total of thirty-five such experiments, however, only two possibly positive results were obtained. In one case, after 14 days, the number of 'cysts' present in the coelomic fluid appeared to be less than originally, but this could not be confirmed with certainty, and no 'cysts' could be found in the sea water around the animal. In another, it appeared that after 10 days the number of nuclei in the microschizonts had increased, but this again was difficult to verify. In none of these experiments did any major change in the pattern of infection occur during the period over which the animals were kept alive.

(2) *Hatching experiments*

At the beginning of this work I was particularly anxious to determine the form of body emerging from the 'cyst', for this, I considered, might give a clear indication of the affinities of the parasite. During the period 1948–51, as suitable material became available, various methods of liberating the 'cyst' contents in a living condition were tried; these are briefly listed below. In these experiments especially, the paucity of material limits the value of the results; again it is possible that in some instances the few 'cysts' dealt with were immature, and that the methods might have succeeded with different samples.

Attempts to remove the contents from the 'cysts' by pressure caused only a slight distortion of the wall and contents, and produced no other result. Later, samples of suitably infected coelomic fluid were treated with the following solutions, in hanging-drop preparations:

(1) Fluid withdrawn by pipette from the gut of *P. minutum*. The fineness and coiled nature of the

* It seems possible that *Selysina* might also have this type of life history, in view of the two kinds of capsules and merozoites; possibly Duboscq's 'spores' were in fact encysted gamonts, and the 'sporozoite' a developing gamont.

gut makes such withdrawal difficult, and it is improbable that the fluid so obtained contained much digestive juice.

(2) Fluid obtained by grinding up the guts of several *Phascolosoma* in sea water.

(3) Fluid obtained by grinding up the guts of *Pygospio* spp. in sea water.

(4) Fluid withdrawn from the stomach and intestine of a Blenny (*Blennius* sp.).

(5) Solutions of various concentrations of commercial extracts of pepsin, trypsin, amylase and lipase.

None of these solutions had any effect at all on the 'cysts'. In an attempt to alter the pH of the fluids, it was noticed that hydrochloric acid, in concentrations of 0·3 % or more, had an immediate effect on the cyst wall, which swelled very rapidly, became transparent and disappeared; this drastic action of course destroyed the contents of the 'cyst'. Lower concentrations of the acid had no effect on the wall of the 'cyst', even after 5 days, though it appeared that the contents were dead.

In view of the similarity of this effect of hydrochloric acid with its known effect on nematode larval skins (see Lapage, 1933), I tried using solutions of 'Milton', which has also been used to exsheath nematode larvae. This gave the most hopeful results, for solutions of concentration of 1/150, or stronger, caused the cyst wall to soften and disappear very rapidly. A 1/175 solution was the lowest concentration which affected the wall, but the contents of the 'cyst' were destroyed before the wall was softened. It appeared that the Milton altered the permeability of the wall before softening it, and so disrupted the contents before there was any chance of emergence.

By August 1951 'cysts' were so rare that no further experiments could be performed.

(3) *Infection experiments*

Specimens of *Phascolosoma* which appeared to be uninfected on first examination were left, in a small quantity of sea water, with coelomic fluid containing 'cysts' or capsules, in an attempt to infect them experimentally. (It was noticeable that the coelomic fluid tended to agglutinate and to stick to the container; in one experiment the 'cysts' were still visible, without apparent change, 16 days after the experiment started.) Further samples of coelomic fluid were then taken at intervals. In only one case was there any difference in the appearance of the coelomic fluid of an animal before and after exposure to the 'cysts': here, after 3 days, a single capsule was observed. It seems likely that this was due to a slight infection which had escaped notice when the first sample was taken, and was not an induced infection. In fifteen other cases, no sign of a new infection could be found, even in longitudinal sections of the animals.

Three specimens of *Pygospio* and one of *Polydora*, which were collected from the same locality as the *Phascolosoma*, were similarly exposed, for 10 days, to infected coelomic fluid; again no trace of the parasite could be found in their bodies.

VII. SUMMARY

1. A description is given of a sporozoan parasite occurring in the coelomic fluid of *P. minutum*.

2. The four stages described include two types of schizonts (each enclosed in a tough envelope), merozoites, trophozoites, and encysted gamonts. The schizonts occur in the haematids of the coelomic fluid, the 'cysts' in amoebocytes.

3. The life history and possible affinity of the parasite with the Haemogregarinidea are discussed.

4. Attempts to verify the suggested life history by experimental means are described.

I wish to put on record my very sincere thanks to Prof. D. L. Mackinnon for her many suggestions and her continuous help and encouragement with this work. I am also indebted to the University of London for the use of the University table at the Plymouth Laboratory in 1947, 1949, 1950 and 1951.

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THE CHEMOTHERAPY OF *EPERYTHROZOON COCCOIDES* (SCHILLING, 1928)

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(With 1 Figure in the Text)

Eperythrozoon coccoides is a pleomorphic blood parasite of white mice. The infection is usually latent but can be evoked by splenectomy; the parasites then appear as coccoid or ring-shaped bodies clustered on the surface of erythrocytes or lying free in the plasma. In heavy infections there is considerable destruction of erythrocytes, and the infected mice occasionally die of the resulting anaemia. Eperythrozoa have been described from many other hosts. *E. wenyoni* causes a mild disease in cattle, *E. ovis* causes a more severe disease in sheep, and *E. suis* has been incriminated by Splitter (1950*a*) as the cause of icteroanaemia of swine, a disease which is widespread in the United States of America.

This paper describes the testing of a wide range of chemotherapeutic agents against *E. coccoides* in white mice. No outstandingly active compounds have been found, but it is hoped that the results may help in determining the correct taxonomic position of *Eperythrozoon*.

METHOD OF TRANSMISSION AND ENUMERATION OF PARASITES

Most of the mice bred at this Institute carry latent infections of *E. coccoides*, but such infections frequently vary in intensity, and the pre-patent period may vary from 2 to 10 days after splenectomy. Heavy infections invariably occur 2 days after splenectomy if mice are first inoculated intraperitoneally with 0·2 c.c. of a 1:100 dilution of heavily infected blood in citrate saline.

In routine examinations thin, dry blood films are fixed with methyl alcohol, dried, and then stained with 10% Giemsa solution in phosphate buffer at pH 7·2 for 1 hr. Eperythrozoa are then stained a pink-purple colour. To estimate the degree of infection the number of parasites per 20 erythrocytes is counted, those free in the plasma and those situated on erythrocytes being recorded separately. For accurate counts, five such areas are examined and an average taken. For chemotherapy experiments, where presence or absence of eperythrozoa is the chief concern, the total number of eperythrozoa in one representative area of 20 erythrocytes is counted and the degree of infection is recorded as follows:

Light infection—up to 5 eperythrozoa per 20 erythrocytes.

Medium infection—5–50 eperythrozoa per 20 erythrocytes.

Heavy infection—more than 50 eperythrozoa per 20 erythrocytes.

METHOD AND RESULTS OF (*a*) FOUR-DOSE AND (*b*) SINGLE-DOSE TESTS

(*a*) Mice which weighed between 13 and 20 g. were infected with *E. coccoides*, as described above. They were kept for 1 or 2 days so that the infection should become well established and were then treated once daily (occasionally twice daily, as shown below) for 4 days with approximately the maximum tolerated dose of drug. They were splenectomized under ether anaesthesia on the second day of treatment several hours after being dosed. By this method mice were treated with drugs both when the infection was latent and when eperythrozoa were numerous in the blood.

Blood films were taken for examination after the last dose, i.e. on the second day after splenectomy. Controls were always heavily infected on this day. Mice with negative blood films were subsequently examined thrice or twice weekly until the infection became patent or for a further 4 weeks. When treated infections became patent they resembled the untreated infections both in the heaviness and the course of infection.

The protocol of a typical experiment is given in Table 1.

(*b*) Drugs which were active in the four-dose test were then tested by a single-dose method at different dose levels. Mice were treated on the second day after inoculation. They were splenectomized 2 days later and blood films were examined thrice weekly from the second day after splenectomy until infection became patent.

In all chemotherapeutic tests three or four mice were used for each drug or for each dose level of a drug. Each group of three or four mice was kept in

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a separate cage to prevent the accidental transmission of eperythrozoa by lice or other means.

Active drugs. Drugs are considered active if no eperythrozoa were seen in blood films from treated mice on the second day after splenectomy. Table 2 shows the dose schedules of active drugs and also the day on which infection became patent, measured from the date of splenectomy. Very slight activity was detected in tryparsamide, chloromycetin, alloxan, and the polyoxyethylene ether (D 2) which is active against *Mycobacterium tuberculosis* (Cornforth, Hart, Rees & Stock 1951). Stibosan was moderately active. Neoarsphenamine, aureomycin and terramycin were very active when four doses were given. Blood films from the mice treated with neoarsphenamine remained negative for 28 days after treatment, and at that time the blood of the treated mice did not infect uninfected splenectomized mice. It is probable, therefore, that the treated mice had been completely cured. The mice treated with aureomycin or terramycin developed acute blood infections 3 or 4 weeks after treatment.

A single dose of neoarsphenamine was more effective than a single dose of either aureomycin or terramycin. Aureomycin, 2·5 mg., and terramycin, 5·0 mg., both delayed infection for 7 days; i.e. aureomycin is approximately twice as active as terramycin against *Eperythrozoon coccoides*.

Table 1. *Specimen protocol of a* 4*-dose chemotherapeutic test against* Eperythrozoon coccoides

(Mice were infected on day 1, treated with drugs on days 3, 4, 5 and 6, and splenectomized on day 4.)

Mouse	Drug	Daily dose (mg./20 g. intraperitoneally)	Blood examinations. No. of eperythrozoa per 20 erythrocytes						
			Day 6	Day 8	Day 11	Day 14	Day 17	Day 21	Day 24
1	*p*-Aminosalicylic acid	4·0	142	34	31	26	28	10	5
2			155	91	28	27	21	36	2
3			133	53	45	23	17	15	6
4	Stibosan	2·0	0	0	0	69	18	15	18
5			0	0	0	22	42	20	42
6			0	0	0	0	64	26	30
7	Control	—	148	132	39	22	19	8	6
8			110	62	56	18	12	5	2

Table 2. *The activity of drugs against* Eperythrozoon coccoides, *as determined by the length of the prepatent period after splenectomy*

Drug	Dose mg./20 g. mouse intraperitoneally	No. of doses	Prepatent period in days (splenectomy on day 0)
Neoarsphenamine	0·1	1	2 (inactive)
	1·0	1	5
	2·0	1	14
	2·0	2	19
	2·0	4	> 28
Aureomycin	0·01	1	2 (inactive)
	0·1	1	5
	1·0	1	5
	2·5	1	7
	2·0	4	21–28
Terramycin	1·0	1	2 (inactive)
	2·5	1	4
	5·0	1	7
	2·0	4	21–28
Tryparsamide	10·0	4	5
Stibosan	2·0	1	2 (inactive)
	2·0	4	11
Chloromycetin	10·0	1	2 (inactive)
	10·0	4	4
Alloxan	1·0	4	5
D 2, polyoxyethylene ether	25·0	4	4 (in 50 % of mice)
Controls	—	—	2

Inactive drugs. Drugs were considered inactive if, after the four-dose test, heavy blood infections occurred in the treated mice on the second day after splenectomy. Table 3 shows the drugs which proved to be inactive, together with a list of the micro-organisms against which each drug is an effective chemotherapeutic agent. Where no separate reference is given, reports of activity of the various drugs can be obtained from Findlay (1950, 1951).

TESTS AGAINST ACUTE INFECTIONS

The speed of action of neoarsphenamine against eperythrozoa has frequently been reported. Thus, neoarsphenamine (neosalvarsan) removed *E. ovis* from the blood of sheep in 15 min. (Neitz, 1937), *E. wenyoni* from cattle in 30 min. (Neitz, 1940), and *E. suis* from swine in 2–6 hr. (Splitter, 1950*b*). However, in each case the disease recurred after treatment.

Neoarsphenamine (2·0 mg./20 g. mouse), aureomycin (3·0 mg.), alloxan (1·0 mg.) and dimidium bromide (1·0 mg.) were injected intraperitoneally into mice with heavy blood infections of *E. coccoides*, the numbers of eperythrozoa being between 60 and 160 per 20 erythrocytes. Blood films were examined at 15 min. intervals for the first hour and then at

2, 3, 5, and 24 hr. No change in the number of eperythrozoa was detected after any of the drugs except neoarsphenamine. Fifteen minutes after treatment with neoarsphenamine the number of eperythrozoa had already decreased, and it dropped steadily until after 2 hr. there were only from 1 to 10 parasites per 20 erythrocytes, and 24 hr. after treatment no eperythrozoa were detected. As no rapid action was discerned in aureomycin or alloxan by this method, and as untreated infections undergo considerable fluctuations in numbers, this test against acute infections was not considered practical for routine screening purposes.

of eperythrozoa have been recorded as percentages of the number which were present in blood films before treatment commenced.

DISCUSSION

Comparison of present results with previous experiments

Vassiliadis (1930–1) claimed that a single dose of neoarsphenamine (2·5 mg./15–20 g. mouse) sterilized mice infected with *E. coccoides*, although infection recurred in 15–20 days after sulpharsphenamine (2·5 mg.). Wigand & Peters (1952) found that the

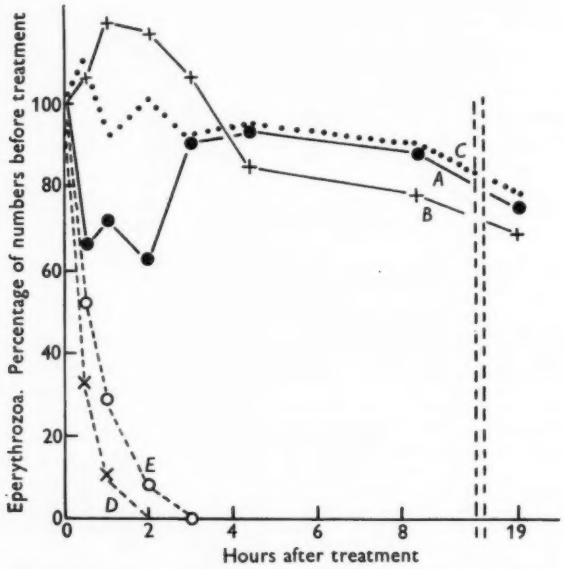

Fig. 1. The action of neoarsphenamine against *E. coccoides* and its antagonism by dimercaprol (BAL). Mice *A*, ●—●, and *B*, +—+, received BAL (2·0 mg. × 2) + neoarsphenamine (0·3 mg.). Mouse *C*, ···, BAL (2·0 mg. × 2). Mice *D*, ×—×, and *E*, ○– –○, neoarsphenamine (0·3 mg.).

Dimercaprol (BAL) antagonized the action of neoarsphenamine upon heavy blood infections of *E. coccoides* in which there were initially between 90 and 150 eperythrozoa per 20 erythrocytes. Infected mice were treated with BAL (2·0 mg./20 g. mouse, subcutaneously, in arachis oil) 1½ hr. before being treated with neoarsphenamine (0·3 mg./20 g. mouse, intraperitoneally) and again 30 min. after treatment. It can be seen from Fig. 1 that the number of eperythrozoa in mice *A* and *B*, which received both BAL and neoarsphenamine, fluctuated similarly to the number in mouse *C*, which received BAL alone, whereas in mice *D* and *E*, which had received only neoarsphenamine, the number of eperythrozoa decreased very rapidly and blood films appeared negative after 3 hr. In Fig. 1 the numbers

infection recurred in 10–20 days in a number of mice treated with neoarsphenamine (2·5 mg. subcutaneously). Reports with sheep (Neitz, 1937), cattle (Neitz, 1940) and pigs (Splitter, 1950*b*) show that doses of neoarsphenamine which controlled the acute infection did not eliminate the disease and the treated animals remained carriers of eperythrozoa. An arseno-antimony compound, Std. 368 B (for formula see Schmidt, 1942), was as active as neoarsphenamine against *E. ovis* (Neitz, 1937). *E. ovis* was also sensitive to the action of acaprin (Delpy & Rafyi, 1938). Vassiliadis (1930–1) found that infections of *E. coccoides* were only delayed for 4 or 5 days by tryparsamide (2·0 mg.), and were unaffected by tartar emetic (0·25 mg.), quinine sulphate (5·0 mg.), suramin (5·0 mg., as 'Germanine') or

stibosan (2·5 mg.). Splitter (1950*c*) found that *E. suis* was unaffected by sodium cacodylate, antrycide or piroplasmin.

Table 3. *Dose schedules of drugs found inactive against* Eperythrozoon coccoides

Drug	Dose mg./20 g. mouse. Daily × 4, intraperitoneally	Parasites susceptible to action of drugs
Hetrazan	10·0	Microfilariae
Fuadin	10·0	Schistosomes
Tartar emetic	0·25	Schistosomes; trypanosomes
Suramin	4·0	Trypanosomes
Antrycide	0·2	Trypanosomes
Dimidium bromide	0·5	Trypanosomes
Acriflavine	0·4	Trypanosomes; Babesia
Stilbamidine	1·0	Trypanosomes; Babesia; Leishmania
Acaprin	0·05	Babesia; *Eperythrozoon ovis* (Delpy & Rafyi, 1938)
Quinine	5·0	Malaria parasites
Mepacrine	2·0	Malaria parasites
Proguanil	0·2	Malaria parasites
Daraprim	1·0	Malaria parasites
Diaminodiphenyl-sulphone	3·0 (b.i.d.)	Malaria parasites; *Mycobacterium leprae*
Sulphadiazine	2·0	Malaria parasites; bacteria; toxoplasma
Methylene blue	1·0	Malaria parasites; *Anaplasma marginale*; *Rickettsia mooseri*
p-Aminobenzoic acid	10·0 (b.i.d.) (orally)	*Rickettsia mooseri* (van den Ende *et al.* 1946)
V. 147*	5·0 (b.i.d.)	
V. 186*	5·0 (b.i.d.)	
V. 238*	10·0 (b.i.d.)	
V. 262*	5·0 (b.i.d.)	
p-Aminosalicylic acid	4·0	*Mycobacterium tuberculosis*
Penicillin	25,000 units	Bacteria
Streptomycin	10·0	Bacteria

**Note.* V. 147 is *p*-Sulphonamidobenzamidine hydrochloride; V. 186 is *p*-Sulphonamidobenzamidoxime hydrochloride; V. 238 is *p*-Sulphonamidobenzamidoxime ureide; V. 262 is *p*-Sulphonhydroxylamidobenzamidoxime base.

In the present experiments, infections of *E. coccoides* became patent within 14–19 days when only one or two doses of neoarsphenamine were given. Mice remained uninfected for 28 days after the four-dose test, in which the drug was given for 2 days before and 2 days after splenectomy. Stibosan, a compound containing antimony, was moderately active against *E. coccoides*; it was considerably more active than tryparsamide. Tartar emetic, quinine sulphate, suramin, antrycide and acaprin were all inactive.

Antibiotics were tested against *E. coccoides* by Wigand & Peters (1952). A single dose of chloromycetin (5·0 mg., subcutaneously) was inactive. A single dose of aureomycin (5·0 mg., subcutaneously), when given on the day of splenectomy, delayed the infection for 2–3 weeks; the same dose of aureomycin, when given to mice with acute blood infections, caused the eperythrozoa to disappear within 24 hr., but the infection recurred within a week in more than half of the treated mice. In the present experiments slight activity was detected in chloromycetin by the four-dose test. Single doses of aureomycin (2·5 mg., intraperitoneally) or terramycin (5·0 mg.) delayed infection for 7 days, although no activity was detected in aureomycin (2·0 mg.) when tested against a heavy blood infection. Four doses of aureomycin or terramycin delayed infection for 21–28 days. These antibiotics are therefore not as effective as neoarsphenamine in controlling infection; in each case the doses which must be given approach the toxic limit. The value of giving a course of at least four doses rather than a single dose has been proved with all the active drugs.

Sensitivity of Eperythrozoon coccoides *to drugs as an indication of its taxonomic position*

Eperythrozoon was considered by Neitz, Alexander & Du Toit (1934) to be a protozoon, and by Mesnil (1929) to be a bacterium. It is very sensitive to the action of arsenicals but is not affected by other drugs which are active against malaria parasites, trypanosomes or piroplasms, nor by sulphonamides, penicillin or streptomycin, which are active against many bacteria.

In its susceptibility to drugs *E. coccoides* closely resembles *Haemobartonella muris*, which is also sensitive to the action of neoarsphenamine (Mayer, Borchardt & Kikuth, 1927; Vassiliadis, 1930–1), stibosan and Std. 386B (Uhlenhuth, 1931; Uhlenhuth & Seiffert, 1933), tryparsamide (Mayer *et al.* 1927), aureomycin and terramycin (Wigand & Peters, 1950; Stanton, Laskowski & Pinkerton, 1950) and alloxan (Laskowski, Stanton & Pinkerton, 1951). *E. coccoides* was only slightly susceptible to tryparsamide and alloxan.

It resembles both the Rickettsiae and the Lymphogranuloma-Psittacosis group of viruses in being sensitive to aureomycin and terramycin and slightly sensitive to chloromycetin, but differs from the lymphogranuloma virus in being unaffected by sulphonamides, and from the rickettsiae of murine typhus fever (*Rickettsia mooseri*) in being unaffected by methylene blue, *p*-aminobenzoic acid or the compounds V. 147, V. 186, V. 238 and V. 262 (see Table 3 for formulae). There are conflicting reports on the activity of arsenicals in several infections with rickettsiae or the large viruses; neoarsphenamine probably has not the same rapid action against these micro-organisms as against eperythrozoa.

Tyzzer & Weinman (1939) considered that *Bartonella bacilliformis* showed affinities with the pathogenic rickettsiae because of its development in vascular endothelium and its morphology *in vitro* and *in vivo*. They suggested that these micro-organisms, together with *Haemobartonella*, *Eperythrozoon* and some others, formed an interrelated group. Weinman (1948), in *Bergey's Manual*, grouped the families Rickettsiaceae, Bartonellaceae and Chlamsdozoaceae under the order Rickettsiales; the family Bartonellaceae consisted of the genera *Bartonella*, *Haemobartonella*, *Grahamella* and *Eperythrozoon*. The chemotherapeutic studies on *Eperythrozoon coccoides* have emphasized the close relationship between this organism and *Haemobartonella muris*, and also the similarities and dissimilarities between these parasites and the Rickettsiae. Until further work has been carried out on these diverse micro-organisms the classification in *Bergey's Manual* must stand as a useful, although possibly over-simplified, indication of interrelationships.

SUMMARY

In tests for chemotherapeutic activity, mice were infected with *Eperythrozoon coccoides*, treated with drugs for 2 days, splenectomized, and treated with drugs for a further 2 days. Blood films were examined from the second day after splenectomy, when control mice showed heavy blood infections.

Neoarsphenamine, aureomycin and terramycin were active.

Tryparsamide, chloromycetin, alloxan and polyoxyethylene ether, D 2, were slightly active.

Many drugs, listed in Table 3, were inactive.

Neoarsphenamine cleared *E. coccoides* from the blood very rapidly. This action was inhibited by dimercaprol (BAL).

E. coccoides closely resembles *Haemobartonella muris* in its sensitivity to drugs. Both micro-organisms probably have affinities with the Rickettsiae.

I wish to thank Dr F. Hawking for his helpful criticism of this work, and Miss Moira Cameron for her technical assistance.

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THE IMMATURE STAGES OF *IXODES FRONTALIS* PANZER 1795

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(With 8 Figures in the Text)

When reviewing the status of *Ixodes frontalis* Panzer in an earlier paper (Arthur, 1952) the reference to *Acarus* (*Ixodes*) *turdi* Scopoli was not included, for the description might well apply to any tick. This is equally true for Panzer's description, and this name has been retained through popular usage. Neumann (1899) was the first worker to present a fairly clear account of this species but, as his host range shows, it may well have been clouded by reference to *Ixodes brunneus* Koch. The available descriptions refer to the female. Larvae have hitherto been neglected or else described as similar to the female, with the addendum that 'in the larva the scutum is as broad as long'. Examination of ticks from a large number of birds has convinced me that in the past the immature stages of many species of ticks have, on this basis, been incorrectly determined. This may possibly be one reason for the suggested rarity of records of the immature stages of *I. frontalis*. In the present paper the larva of this species has been described. This description has been based on larvae sent to me by Mr John Ash.

Capitulum (Fig. 1). Dorsal surface length 0·089 mm.; width of basis 0·055 mm.; lateral profile lines curved. The front margin of the basis between the palps and hypostome forms a forwardly directed protuberance. Posterior margin straight or a little sinuous, salient. Cornua absent, dorsal ridge drawn out laterally. Surface punctate. Palps long and broad, but narrowing distinctly at the base of segment II, inner profile lines more or less straight, outer edges gently curved. Suture between articles II and III faintly indicated on outer margin. Combined length of segments II and III, 0·053 mm. Hairs long, but not numerous.

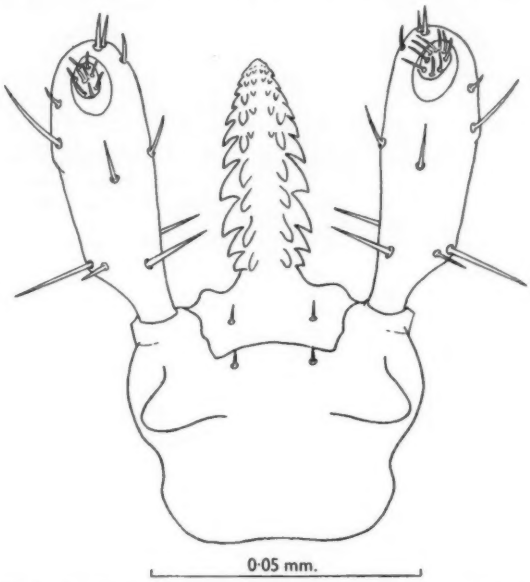
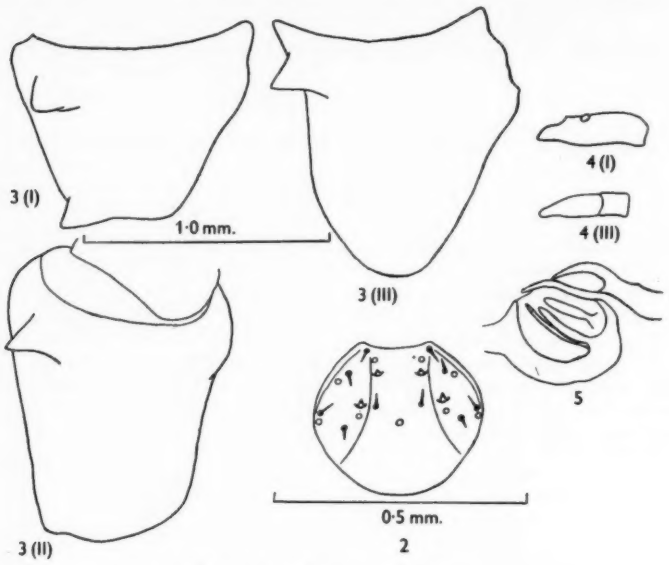


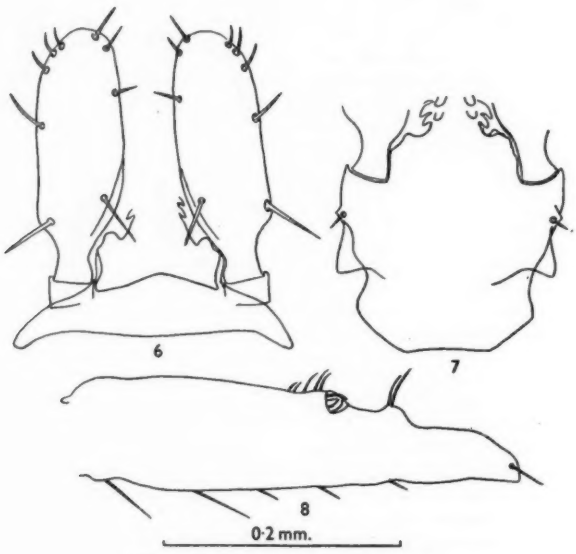
Fig. 1. *Ixodes frontalis*. Larva. Capitulum viewed ventrally.

In ventral view basis broad, becoming narrower posteriorly, terminating in a more or less straight edge, slightly constricted behind the auriculae which appear as lateral extensions usually with a rounded apex.



Figs. 2–5. *Ixodes frontalis*. Larva.

Fig. 2. Scutum to show bristles, hastiforme and auriforme sensillae.
Fig. 3. Coxae I to III.
Fig. 4. Tarsus I and tarsus III (same scale as Fig. 2).
Fig. 5. Haller's organ showing four sensillae, of which the most dorsal passes through the aperture and curves down (same scale as Fig. 1).



Figs. 6–8. *Ixodes frontalis*. Nymph.

Fig. 6. Capitulum seen dorsally.
Fig. 7. Capitulum seen ventrally.
Fig. 8. Tarsus I.

Hypostome (Fig. 1). Length about 0·04 mm. Profile lines curved, with pointed tip. Denticles arranged from base to apex in 2/2 files for 4 rows, 3/3 files for 3–5 rows, thereafter very small, 10–12 lateral denticles conspicuous; lateral denticles larger than median denticles.

Scutum (Fig. 2). Length 0·3 mm.; width 0·33 mm.; i.e. broader than long (cf. Neumann, 1899). Emargination slight. Ovate in shape, widest about the middle, broadly rounded behind. Cervical grooves as valleys separating the lateral and median areas and extending back nearly to postero-lateral borders. Lateral carinae present. Surface finely punctate. Hastiforme sensillae, auriforme sensillae and bristles arranged as shown in Fig. 2 (compare with fig. 24, Arthur, 1953).

Legs. Long and large. Hairs numerous and conspicuous. Tarsus I broad and long, tarsus III relatively thinner and shorter. Length of tarsus I, 0·225 mm.; length of tarsus III, 0·183 mm. (Fig. 4). All tarsi taper to apex, but there is a slight hump on tarsus I immediately in front of anterior pit of Haller's organ.

Coxae (Fig. 3). All coxae mildly convex, shining. Coxa I with prominent internal and external spines, both of about same length; coxae II and III have the external spurs more pointed and longer than in coxa I.

The capsule of Haller's organ (Fig. 5) contains 4 sensillae of which the dorsal sensilla is broadest, is curved and penetrates through the capsular aperture. This bears a very close resemblance to the female organ, except in the smaller number of bristles, and as shown elsewhere (Arthur, unpublished data) the capsule is of diagnostic value in identifying all stages of the genus *Ixodes*, in Britain at least, and may possibly have a wider application.

Only one nymph of *I. frontalis* was available for examination, and unfortunately the hypostome was broken during removal from the host.

In this specimen the postero-lateral angles of the dorsal ridge of the basis project laterally and curve gently back (Fig. 6). Prominent auriculae arise from the margins of the basis (Fig. 7); and a pointed projection with a basal bristle arises in front of each of the auriculae. The basis is gently 'waisted' behind the latter and the postero-lateral margins angled. The legs, including Haller's organ (Fig. 8), resemble those of the female in all respects except size.

Hosts. Larvae were collected from a blackbird and two chaffinches, and the nymph from an adult willow warbler. The details are as follows:

Turdus merula merula. Blackbird (1st winter female) from Portland Bird Observatory, Dorset, 13 October 1951). Four larvae.

Fringilla coelebs. Chaffinch (male) from Fordingbridge, Hants, 26 January 1951. One larva.

F. coelebs (female) from Fordingbridge, Hants. One larva.

Phylloscopus t. trochilus L. Willow warbler (on head of adult female) from Skokholm Bird Observatory, Pembs, 13 April 1952. One nymph.

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STUDIES ON THE CROSS-TRANSMISSION OF POLYHEDRAL VIRUSES: EXPERIMENTS WITH A NEW VIRUS FROM *PYRAMEIS CARDUI*, THE PAINTED LADY BUTTERFLY

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(With Plates III–VIII)

INTRODUCTION

Up to the present time the polyhedral viruses of insects have been considered to be extremely specific in their infectivity, and cases of cross-infection, even between closely related species, were regarded as rare occurrences. Recently, however, we have recorded results which suggest that there is no such specificity of infection (Smith & Xeros, 1952). In the following paper we describe the results obtained in an extensive series of cross-inoculations among a heterogeneous collection of lepidopterous larvae, selected entirely at random. The results obtained appear at first sight to be compatible with one clear-cut explanation of straightforward cross-infections with differential multiplication of two different viruses in different hosts. Other complications, however, cannot be excluded and one or two possible situations are discussed later in the paper.

Attention is largely confined to inoculations carried out with an undescribed virus, or viruses, obtained from larvae of *Pyrameis cardui*, the Painted Lady butterfly, though other cross-inoculation experiments may be referred to by way of illustration.

Much of the significance of this paper hinges on an earlier discovery (Smith & Wyckoff, 1951) that there exist at least two distinct types of polyhedra, the difference being revealed when the polyhedra are treated with weak alkali and observed with the electron microscope. These two types we refer to as the '*membrane* type', in which the crystal is completely dissolved by the alkali, leaving behind a collapsed membrane usually containing a varying number of virus rods, first observed by Bergold (1947), and the '*shell* type' in which a shell or skeleton is left by the alkali, usually containing numbers of spherical holes with which in some cases a spherical virus is apparently associated. Two other significant facts which play a large part in these studies are the discoveries, first that the shell-type polyhedral body readily picks up stains and so can be easily differentiated from the membrane type on the optical microscope, and secondly that both types may occur in the same caterpillar (Smith, Wyckoff & Xeros, 1953). Furthermore, we know now that there exist two entirely different kinds of polyhedral diseases. In one, here referred to as the nuclear type for brevity, polyhedra arise in the cell nuclei and are confined to the fat body, skin and tracheal tissues. In the other, the cytoplasmic type, the polyhedra arise, not in the nucleus, but in the cytoplasm and are confined to the alimentary canal, or midgut, which may become choked with a solid plug of polyhedra (see *Bombyx mori*, Pl. IV, fig. 6). The evidence presented in this paper seems to confirm that the polyhedra in the cytoplasmic type of disease are of the staining or shell type as shown by Xeros (1952). It should be mentioned that a well-developed gut polyhedrosis is readily recognized macroscopically. As with a nuclear gut polyhedrosis (Balch & Bird, 1944), in the cytoplasmic disease the midgut becomes very milky and loses its translucence. The gut also becomes very friable in character, instead of gelatinous as it is in the healthy larva.

In presenting the results of these studies, we have thought it better to deal with the cross-transmission experiments seriatim, describing for each case the kind of disease resulting and the type of polyhedral body formed. A number of back-cross transmissions are also described.

MATERIAL AND METHODS

The large number of caterpillars used in these experiments were reared mainly in three types of containers. The newly emerged or small larvae were kept in glass-topped metal boxes from which they were transferred when necessary to glass jars with screw-topped metal lids. The lids were pierced with holes, about a cm. in diameter, and a sheet of thick filter-paper was fitted underneath to prevent aerial contamination and the escape of the larvae. When large numbers of larvae, 100 or more, were employed, a metal cage was used. This was fitted with a groove in front in which a sliding glass panel was introduced and there was a circular opening at the back with

a collar round which was tied a sheet of filter-paper or muslin to give ventilation. All these containers were sterilized by dry heat in an electric oven.

Most of the inoculation was carried out by feeding; the foliage was heavily contaminated with the desired polyhedra and allowed to dry before being given to the caterpillars.

The different species of larvae, when diseased, were all examined in a similar manner. A blood smear was first made on a microscope slide, dried over the bunsen using a minimum of heat and stained with methylene blue or Giemsa. The latter stain gives the best results. Dissection of the caterpillars, followed by smearing of the separate tissues so far as possible, was carried out in most of the work. If these preliminary tests warranted it, the caterpillar was then cut into two or three pieces, fixed in Carnoy's Fluid, embedded in wax and sectioned. The observations of the polyhedra on the optical microscope were in nearly every case checked on the electron microscope, although in this study we have not examined any sections by this means. For examination in the electron microscope, drops of polyhedral suspensions were put on formvar-covered grids and allowed to dry. They were then treated with sodium carbonate in strengths varying from 0·5 to 5 % for periods of ¼–5 min., shadowed with palladium and photographed on a Metropolitan Vickers EM_3 100 kV. microscope at 75 kV. The electron micrographs (except Pl. VIII, figs. 15 & 16) were taken by Miss Sheila Vernon-Smith. Mr S. Frey took the photographs on the optical microscope.

Pyrameis cardui (Painted Lady Butterfly)

Infected caterpillars of this insect were the only source of inoculum for the experiments described in this paper.

Some very young larvae, about 24–48 hr. old, were purchased from a dealer and, on arrival or shortly after, several were observed to be dying. Smears were made from these moribund larvae and stained with methylene blue. The insects were found to be infected with a polyhedral disease in which were observed large quantities of rather small and uniform polyhedra of the non-staining or membrane type. However—and this is important in view of the rest of the experiments—here and there could be seen one or two polyhedra which had readily taken up the stain. When treated with alkali and observed on the electron microscope, the great majority of the polyhedra were found to have a well-developed membrane and numbers of rod-shaped virus particles could be seen (Pl. VII, fig. 11).

In addition there were a few apparent shell-type polyhedra; these did not show the well-defined 'honeycomb' appearance, which can be seen in *meticulosa*, for example, but as they occur throughout the various tests with many different species we assume that these are in fact shell-type polyhedra.

Another important point in the polyhedral disease of *P. cardui* is the fact that the larvae were infected with an apparently dual disease and, since the infected larvae had only recently emerged from the egg, it must be assumed that both viruses had been passed from parent to offspring. This fact is relevant in the consideration of latent viruses.

In order to increase the amount of inoculum, more healthy *cardui* larvae were infected with the virus from the original larvae. They were found to be very susceptible and death ensued usually within 5 or 6 days of infection. In nearly every case the disease induced was a typical nuclear polyhedrosis, but in a few instances dissection of the infected larvae showed the gut to be full of the cytoplasmic shell-type polyhedra as well.

None of this stock of *cardui* larvae developed a natural infection with polyhedral disease.

Aglais urticae (the Small Tortoiseshell Butterfly)

The caterpillars of this butterfly are very susceptible to infection. A first batch of twelve half-grown larvae were given nettle foliage contaminated with *cardui* polyhedra on 15 July; on 26 July, 11 days later, all the larvae were dead of polyhedral disease. The bodies were limp and ruptured at a touch, the skin often bursting of its own accord. There seems to be an unusually heavy development of polyhedra associated with the virus. On 6 August a second batch of 100 larvae were infected. Eight days later, one or two larvae were found dying of the disease, but the rest of the batch looked fairly normal. The next morning, however, the change was quite dramatic, more than 90 % of the larvae were dead and hanging down in the manner characteristic of death from this cause. Some of these larvae are shown in Pl. III, fig. 1, and in one or two instances the head of the hanging caterpillar appears as a black, shining sphere. This is a drop of the liquefied body contents which are beginning to run out. The completed process is shown in Pl. III, fig. 2. Only two larvae out of the hundred succeeded in getting as far as the pupal stage and one of those also died. No larvae of *A. urticae* have ever been found by us naturally infected with polyhedral disease and all the very large control stocks of this caterpillar remained healthy. When viewed under the optical microscope the polyhedra appeared similar to those in *cardui*. The electron microscope showed that the vast majority of the polyhedra were of the membrane type with many virus rods. Here and there, however, shell-type polyhedra were also found. No caterpillars dying with a gut polyhedrosis were observed, although the cytoplasmic shell-type polyhedra were also present.

Vanessa io (the Peacock Butterfly)

Fifty half-grown larvae of *V. io* were given foliage contaminated with polyhedra from *urticae* infected from *cardui*. Some days later it was noticed that the larvae were on the whole smaller than the control stocks. It is possible that this was induced by an initial shock due to absorption of the virus which caused the larvae to cease feeding for a time. Ten days after infection, a number of the larvae were dead and, 2 days after this, all but four of the remaining larvae were also dead, hanging limp and flaccid in the usual manner. Three days later all the caterpillars had died of polyhedral disease. In the later stages of infection many of the larvae developed characteristic dull patches on the integument and this was frequently followed by an over-all moistening of the skin, giving the insect an oily appearance. As with *A. urticae* we have never found larvae of *V. io* naturally infected with a polyhedral disease and all the control larvae, numbering over two hundred, remained healthy.

Examination of the infected larvae with the optical microscope showed polyhedra present in the skin, fat body and tracheae but not in the gut. In addition, portions of the gut of infected caterpillars were fixed in Carnoy and sectioned but no polyhedra were observed. On the electron microscope many membrane-type polyhedra with accompanying virus rods were seen and also a few of the shell-type polyhedra.

Pararge megara L. (the Wall Butterfly)

Twelve small larvae of *P. megara* were infected with polyhedra from *urticae* which had been infected from *cardui*. In 6 days four of the larvae were dead of polyhedral disease and in 8 days from the start of the experiment all were dead. Smears examined on the optical microscope showed the great majority of the polyhedra to be of the cytoplasmic or shell type, although a few non-staining polyhedra could be observed here and there. Sections of these caterpillars fixed in Carnoy and stained with Giemsa showed the gut cells filled with large staining polyhedra which were confined to the cytoplasm; the nuclei appeared normal and free from polyhedra. Examination of the polyhedra on the electron microscope confirmed these observations. Ninety per cent or more of the polyhedra were shell type, but here and there a few membrane-type polyhedra could be seen. No virus rods, however, were found. It should be noted that the case of *P. megara* is the exact reverse of the conditions found in the three previous butterfly larvae, although the same inoculum was used. In the latter cases, the caterpillars all died of a nuclear polyhedrosis caused by the membrane-type polyhedra with a few shell-type polyhedra also present. The larvae of *P. megara*, however, all died of a cytoplasmic gut polyhedrosis caused by the shell-type polyhedra with a few membrane-type polyhedra also present.

Pararge aegeria L. (the Speckled Wood Butterfly)

Twelve medium-sized larvae of this butterfly were infected with polyhedra from *urticae* ex *cardui*. In 9 days all the larvae were dead of polyhedral disease. Observations of sections on the optical microscope and of the polyhedra on both microscopes revealed a similar condition to that found in *P. megara*.

Heodes phlaeas (the Small Copper Butterfly)

Twenty-four larvae of *phlaeas* were infected with *cardui* polyhedra *ex urticae*. In 18 days all the larvae were dead. Examination of the polyhedra and sections of the larvae on the optical microscope revealed a similar condition of gut polyhedrosis to that found in *Pararge megara* and *P. aegeria*. These polyhedra were not examined under the electron microscope.

Pieris brassicae (the Large White Butterfly)

Two experiments with the caterpillars of the Large White butterfly were carried out. In the first experiment, twenty-four small larvae were given cabbage leaves contaminated with polyhedra from *cardui* ex *urticae*. None of these larvae became infected but pupated normally and the majority gave rise to adult insects. In the second experiment 200 caterpillars were contaminated, this time with polyhedra direct from *cardui*, with the same result.

It is interesting to note that this is the only lepidopterous larva of all the species tested which failed to develop a polyhedral disease.

Bombyx mori (the Silkworm)

On 8 August, two dozen silkworms in the second instar were given mulberry leaves contaminated with *cardui* polyhedra ex *urticae*. On 15 August some of the larvae appeared to be in rather a static condition and to have ceased feeding. No polyhedra, however, were detectable in the blood. On 30 August, just over 3 weeks after infection, three silkworms died of polyhedral disease. On 4 September three more nearly mature silkworms were dead. There seemed to be no polyhedra under the skin, and in the fat cells the nuclei were dark staining and free of polyhedra. In the gut cytoplasm, however, staining polyhedra were present in large numbers. The gut was gorged with an almost solid plug of polyhedra. The whole alimentary canal was dissected out and portions of it fixed and sectioned. In Pl. IV, fig. 7, can be seen part of a section through the gut showing some of the large staining polyhedra. Pl. IV, fig. 6, is a photograph of the interior lumen of the gut showing the masses of polyhedra. On 10 September, it was noticed that one silkworm had failed to make the usual opaque yellow cocoon

but was enclosed instead in a thin semi-transparent envelope. On opening this, the larva was found still alive and apparently normal except for a swollen and turgid appearance. On dissection it was found that this appearance was due to the condition of the gut which was filled almost to bursting point with polyhedra. By inserting a fine pipette into the alimentary canal, it was possible to draw off a white and practically pure suspension of polyhedra. Some of these were treated with sodium carbonate and photographed on the electron microscope. This revealed that they were of the staining shell-type, in keeping with all the other cases of cytoplasmic gut polyhedroses. However, careful search revealed one polyhedral body of the membrane type with some apparent rods nearby. Pl. VIII, figs. 15, 16, show the shell-type polyhedra and one polyhedral body of the membrane type. We are indebted to Dr R. W. G. Wyckoff for these two photographs. Some serial transmissions were also made as follows. Second-instar silkworms were infected with polyhedra from other silkworms which were themselves infected from larvae of *Aglais urticae* infected from *cardui* larvae. Twenty-four to thirty days later, five silkworms died of a typical gut polyhedrosis. No polyhedra of the membrane type were observed.

Phalera bucephala (the Buff Tip Moth)

On 22 August three dozen small larvae of the Buff Tip moth, out of a large number collected in the Cambridge district, were fed on birch leaves contaminated with the *cardui* polyhedra ex *urticae*. On 29 August, 7 days later, all the larvae except two were dead of polyhedral disease and exhibited the characteristic flaccid appearance. Smears made from these larvae showed large numbers of non-staining polyhedra in the nuclei of the fat body. Examination on the electron microscope confirmed that the vast majority of the polyhedra were of the membrane type, though a few shell-type polyhedra were also present. The remaining two larvae continued feeding and appeared normal. However, by 10 September, they were both dead, although they still retained a normal appearance without the flaccid condition characteristic of the other thirty-four larvae. Examination of these two larvae showed that they had died of a gut polyhedrosis. No polyhedra could be found in the blood but the lumen of the gut was filled with masses of staining polyhedra and large numbers could also be observed in the cells of the gut wall when fixed with osmic acid and examined under the optical microscope. A portion of the gut was also fixed in Carnoy's Fluid and sectioned; examination of these sections showed that the nuclei were free of polyhedra but there were large numbers in the surrounding cytoplasm.

None of the control stocks of *bucephala* caterpillars died, and we have never observed a natural infection with polyhedral disease in this species. The infection of *bucephala* is an interesting case because not all the caterpillars, though treated similarly, reacted in the same manner. It makes a good comparison with the infection of the larvae of the angleshades moth, *Phlogophora meticulosa*, and of the larvae of the tropical silk moth, *Philosamia cynthia*.

Phlogophora meticulosa (the Angleshades Moth)

A batch of twelve small larvae of the Angleshades moth were infected with a suspension of polyhedra direct from *cardui*. A few days later a second batch of twenty-four similar larvae were also infected. In 14 days the first larva died of polyhedral disease. Investigation of this larva revealed the interesting fact that it had both a nuclear disease of the fat body and a cytoplasmic disease of the gut. Both kinds of polyhedra were present with a slight preponderance of the shell type; this was demonstrated both on the optical and the electron microscopes. On the latter good photographs of membranes and rods, together with the typical 'honeycomb' skeleton of the shell-type polyhedra, were obtained (Pl. VII, figs. 13, 14). The remainder of the thirty-six larvae continued to die at intervals, but they did not succumb in a batch after the manner of *Vanessa io* and *bucephala*. Most of the remaining larvae died of a cytoplasmic gut polyhedrosis with the staining shell-type polyhedra in great excess. However, several of the larvae also developed the dual type of disease. In these, both kinds of polyhedra were about equally numerous. Serial transmissions were also carried out, in which *meticulosa* larvae were inoculated from *meticulosa* larvae infected from *cardui*. Fifteen larvae out of about twenty died with a cytoplasmic gut polyhedrosis.

Philosamia cynthia (Ailanthus Silk Moth)

Twelve larvae of this tropical silk moth were infected with the *cardui* polyhedra ex *urticae*. After about 3 weeks the larvae commenced to die of polyhedral disease. Here again the reaction was interesting and the larvae did not all develop the same type of polyhedrosis. Tests made of the blood and fat body of one of the first larvae to die showed no nuclear, non-staining polyhedra, but when the gut was opened it was found to be full of cytoplasmic shell-type polyhedra of rather large size. Examination on the electron microscope showed, as was expected, that the shell-type polyhedra were present in excess, with here and there a membrane-type polyhedral body. In these, numerous rods were visible. It is worth noting here that small numbers of spherical particles could be observed; these might conceivably be virus particles liberated from the shell-type polyhedra.

The last larva of *cynthia* to die was a very good

example of the double disease. Smears made of the blood and fat body showed large numbers of rectangular non-staining polyhedra and many nuclei packed with polyhedra were also seen. The larva was then opened and the alimentary canal examined. In this case the cytoplasmic polyhedrosis was well developed only in a middle section of the midgut, which showed up plainly as a milky white patch containing vast quantities of polyhedra. In Pl. III, fig. 3, may be seen a smear from this insect in which the nuclear polyhedra are lying scattered above the deeply stained cytoplasmic polyhedra.

Some of these rectangular nuclear polyhedra were examined under the electron microscope. The usual membranes were found some of which were empty; others, however, were unusually full of virus rods, and it is interesting to note that many of these were in bundles. In Pl. VIII, fig. 19, these bundles, together with many single rods, are well shown.

Arctia caja (the Garden Tiger Moth)

About fifty young *caja* larvae were infected with polyhedra direct from *cardui*; after 9 days they started to die of polyhedral disease. Smears made from these larvae and examined on the optical microscope showed numbers of membrane-type nuclear polyhedra. This was confirmed on the electron microscope which indicated that this type of polyhedra was present in excess; shell-type polyhedra, however, were also present in small numbers.

It is relevant here to note that another stock of older *caja* larvae, obtained from quite a different source, commenced to die about this time from a natural infection with a polyhedrosis. Examination of these larvae on both the optical and the electron microscope showed that *only* cytoplasmic shell-type polyhedra were present. This is significant in view of the fact that the inoculated *caja* larvae developed a nuclear polyhedrosis with the accompanying membrane-type polyhedra in excess.

It was in *Arctia caja* and the related *A. villica* that the existence of shell-type polyhedra was first demonstrated (Smith & Wyckoff, 1950).

Sphinx populi (the Poplar Hawk Moth)

Six small larvae of this species were fed on foliage contaminated with polyhedra from *cardui* ex *urticae*. Within 4 weeks all six larvae were dead of a polyhedral disease. Under the optical microscope only small non-staining polyhedra of the nuclear type were observed. They were not examined under the electron microscope. All the control larvae remained healthy and we have never found a natural polyhedral infection of this species.

BACKCROSS-INFECTIONS

So far, cross-infections into various species have been described including serial passages into *mori* and *meticulosa*, using the original *cardui* virus(es) ex *urticae* and the original *cardui* polyhedral material respectively.

A sample selection of backcross-infections was carried out to establish the transmissibility of the polyhedral viruses resulting from these cross-infections back into the original host, *cardui*, and to establish intertransmissibility of the polyhedral viruses from the secondary hosts. Additionally, an attempt was made to obtain further purification of the nuclear and cytoplasmic viruses from each other, if indeed there were two distinct viruses in the original polyhedral material. The resulting polyhedral material of these experiments was not examined under the electron microscope. A few of these backcross-infection experiments will be briefly described.

Exp. 1. Material of cytoplasmic gut polyhedra from a prepupa of *mori* was used to infect four *cardui* larvae. One died of a typical nuclear polyhedrosis, and no cytoplasmic gut polyhedra were found in it. The other three larvae died of well-developed nuclear and cytoplasmic diseases.

Exp. 2. Polyhedra from what appeared to be an exclusively gut polyhedrosis in a larva of *bucephala*, infected as *mori* above, with *cardui* ex *urticae* polyhedra, were used to infect five *cardui* larvae. All five died in 9–12 days with well-developed nuclear and cytoplasmic polyhedroses.

Exp. 3. Polyhedra were used from the larva of the Wall Butterfly (*Pararge megara*) which as far as could be determined by means of the light microscope, had developed only gut cytoplasmic polyhedra after infection with *cardui* ex *urticae* material. Five *cardui* larvae were fed with the polyhedra and all died in 12–15 days of cytoplasmic gut polyhedroses. No non-staining membrane-type polyhedra were found in the tissues. The above three backcross-experiments were all successful. However, although all the inocula were chosen from caterpillars with well-developed gut polyhedroses in which no membrane-type polyhedra were observed, both types of polyhedra developed in the *cardui* larvae injected from *mori* and *bucephala*. An effective sieving or filtering action was here achieved only by *megara*. It has already been noted that all these inocula when investigated with the electron microscope, showed a few membrane-type polyhedra to be present. It was next decided to infect *bucephala* larvae with polyhedra from *mori* ex *urtica* ex *cardui*, to see first if the viruses from the secondary hosts were intertransmissible, and secondly to compare the results, if successful, with backcross-infections into *cardui*.

Exp. 4. Three *bucephala* larvae in the last instar were fed with the material. All died in 11–16 days of typical cytoplasmic gut polyhedroses. No non-staining membrane-type polyhedra were found.

Exp. 5. The gut polyhedra from one of the above larvae were then used to infect three *cardui* larvae. They died in 2–3 weeks of gut cytoplasmic polyhedroses, no non-staining nuclear polyhedra were found in smears.

Thus, when polyhedra from *mori* were passed directly into *cardui*, both nuclear and cytoplasmic diseases resulted. However, if first passaged through *bucephala*, only the cytoplasmic disease resulted in *cardui*, suggesting sieving or filtering action had taken place in the *bucephala* larva.

Exp. 6. The cytoplasmic gut polyhedra of *mori*, used in Exp. 1, were passed through *mori* again, with the same symptoms resulting, and were then fed to five *cardui* larvae. One larva survived to pupate, the other four died in 11–21 days with well-developed gut polyhedroses. No non-staining polyhedra were found in these caterpillars. So, whereas one passage through *mori* resulted in a double disease on back-inoculation to *cardui*, after two passages the gut cytoplasmic disease alone developed. It seems then that of all the species used, *mori* was one of the most effective in filtering out the membrane or nuclear type polyhedra, whilst *cardui* behaves in the opposite manner.

DISCUSSION

As a basis for the discussion it will be well to recapitulate as briefly and concisely as possible the results obtained with the large series of direct cross-inoculations described in this paper. The original inoculum was obtained from very young larvae of *Pyramels cardui*, the Painted Lady butterfly, and the extremely early stage of these larvae makes it virtually certain that the virus, or more probably viruses, had passed from parent to offspring. The various types of reaction in the inoculated larvae may be classified as follows: (1) Both nuclear and cytoplasmic gut polyhedroses develop in the same larva. (2) A nuclear polyhedrosis alone develops. (3) A cytoplasmic gut polyhedrosis alone develops. Some larvae of the same species, all of which had the same dose of inoculum, may develop both types of disease whilst others may develop either a nuclear or a cytoplasmic gut polyhedrosis. For example, one or two caterpillars of *Phlogophora meticulosa* and *Philosamia cynthia*, a tropical silk moth, developed both nuclear and cytoplasmic diseases in the same individual whilst others, and these were in the majority, developed a cytoplasmic gut polyhedrosis alone. On the other hand, the majority of the caterpillars of *Phalera bucephala* developed only a nuclear polyhedrosis, whilst others had only a cytoplasmic disease. It must be emphasized, however, that in every case of an artificial inoculation, whether the disease resulting was a nuclear or cytoplasmic polyhedrosis, *both types* of polyhedra were present. However, the polyhedra characteristic of the disease which develops are always present in an overwhelming majority. It was only when *both* diseases had developed that both types of polyhedra were present in more or less equal numbers. This occurred, as already mentioned, in *meticulosa* and *cynthia*. The most obvious explanation of these results is the existence in the original *cardui* of two viruses, one of which causes a nuclear disease with polyhedra of the membrane type and the other causes a cytoplasmic disease with polyhedra of the shell or staining type. The backcross-inoculations into *cardui* from *mori*, *bucephala* and *megara* further confirm this explanation of the results. So does the intertransmission of virus from *mori* to *bucephala* and its subsequent back infection to *cardui*. On back inoculation to *cardui*, after two transmissions through *mori*, or through *mori* and *bucephala*, gut polyhedra were obtained almost, if not quite, exclusively in *cardui*. Whereas after only one passage through either of these species, and using inoculum from larvae in which only cytoplasmic polyhedra could be detected, both nuclear and cytoplasmic diseases were obtained in the inoculated larvae. Together with the evidence from the electron microscope, these results indicate that in the original *cardui* and *cardui* ex *urticae* material, two distinct viruses were present. This seems a more likely explanation than that only one virus is present which may, according to conditions, give rise to one or other or both of the two types of disease. Further, the evidence supports the view that one virus causes the cytoplasmic gut polyhedrosis with staining-type pitted polyhedra, and that another distinct virus causes the nuclear polyhedrosis of the hypodermis, tracheae and fat body with membrane-type polyhedra. This conclusion is in line with the fact that the well-known polyhedral viruses give rise to nuclear polyhedroses only, of the hypodermis, tracheae and fat body with non-staining membrane-type polyhedra. Furthermore, the polyhedral viruses of *villica*, *caja* and *purpurata* give rise in the host species and in cross-infections to gut cytoplasmic polyhedroses only, with staining type pitted polyhedra (Xeros, 1952).

The backcross-inoculations and cross-intertransmissibility tests which were done, were chosen mainly on the basis of the availability of adequate control stocks of healthy larvae. The fact that all these tests were successful is consistent with the explanation that two distinct viruses were involved which were being truly cross-transmitted and differentially produced in the various hosts. The fact that no negative results were obtained in these tests, as might have been expected if another explanation had been the true one, gives added weight to the above theory of two viruses.

Starting with a mixture of the two types of polyhedra, each of probably different chemical com-

position and with differential rates of multiplication, it was felt that chemical characterization at this stage would be valueless and we were not in a position at the time to carry out antigenic studies. It will, however, be profitable to carry out such chemical and serological characterization on material resulting from cross-infections with viruses known to be constant in their cyto- and histological manifestations.

The explanation of our results given above is consistent with all the facts including the backcross-inoculations. However, it is known (Smith & Wyckoff, 1951) that caterpillars of a given species may be bred for a number of generations and then they may succumb to polyhedral infection under conditions precluding external infection. We have, therefore, to take this fact of latency of viruses into consideration. It might be objected that in the experiments described in this paper, the introduced viruses acted as stimuli for latent viruses already present and that no true cross-infections were obtained. Alternatively, it might be suggested that when two viruses are inoculated into a larva which has already one of them latent within the body, it is only the new virus of the two which enters. The other virus merely serves as a triggering mechanism which stimulates the latent virus into multiplication. Whilst fully admitting this possibility, we nevertheless consider, in the light of the evidence of so many successful backcross and intertransmission experiments, that true cross-infection does take place. Incidentally we might mention in this connexion that none of our silkworm stocks has developed a natural infection with a polyhedrosis during the past 2 years. Moreover, forty larvae of this stock were subjected to fairly strong nitrite treatment as described by Yamafuji (1952) and none of them developed a polyhedrosis. In addition, one would expect a latent virus in silkworms to be of the nuclear membrane type, the only one described in the literature as a natural infection of this larva; yet in our experiments it was the gut cytoplasmic polyhedrosis which usually developed after inoculation. Similarly, *caja* larvae inoculated with nuclear polyhedra from *cardui* developed a nuclear polyhedrosis but the disease resulting in this species from a natural infection has always been a cytoplasmic one. Some interesting facts have been established from this and the previous work, which may be emphasized. Thus, we appear to have two viruses, each of which has its peculiar type of polyhedral body, differing from each other in many essential points. One arises in the cell nucleus of fat body, tracheae and skin tissues, and the other arises in the cytoplasm of the gut. Moreover, an entirely different disease is associated with each of the two types of polyhedra. In addition there is evidence that the virus from the shell-type polyhedra is a sphere compared with the rod-shaped particles in the nuclear or membrane-type polyhedra.

In a previous communication (Smith, Wyckoff & Xeros, 1953) we have shown a peculiar 'honeycomb' structure in the nucleus of the cell which is apparently connected with the mechanism of formation of this type of polyhedral body. Since the cytoplasmic polyhedra do not form in the cell nucleus, there must be some method of formation of which we know nothing.

Much work remains to be done on the factors governing the multiplication of the viruses and on the reasons why some caterpillars develop a gut polyhedrosis and others a nuclear disease.

Obviously many more examples of cross-transmission of viruses could be made and there is probably no limit to the number of species which can be infected. This brings up the possible economic application of this work to the control of harmful insects. It seems feasible to suggest that certain lepidopterous pests of crops and stored products, which have not been observed to develop virus diseases, could now be given a virus from some other unrelated species of insect.

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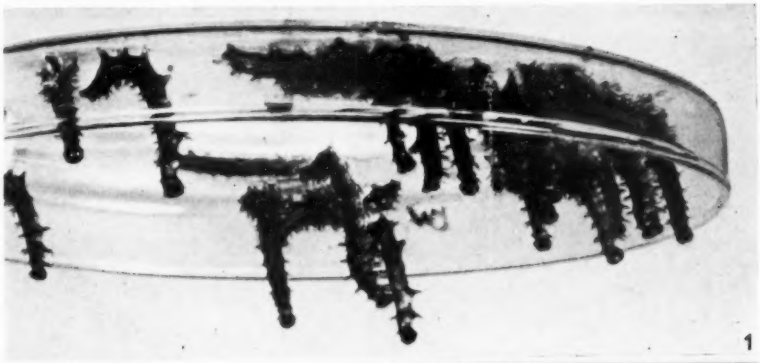
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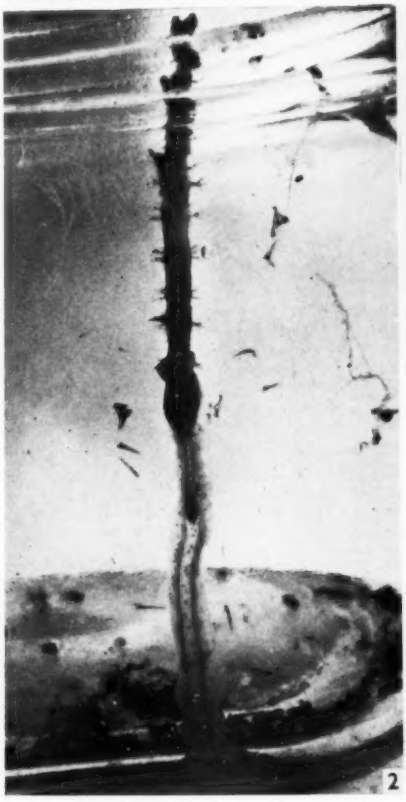
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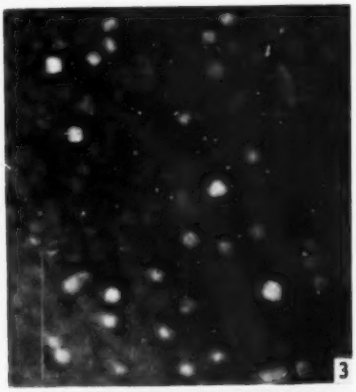
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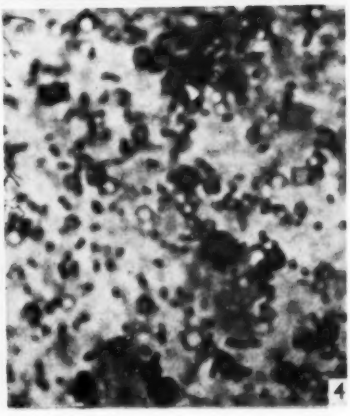
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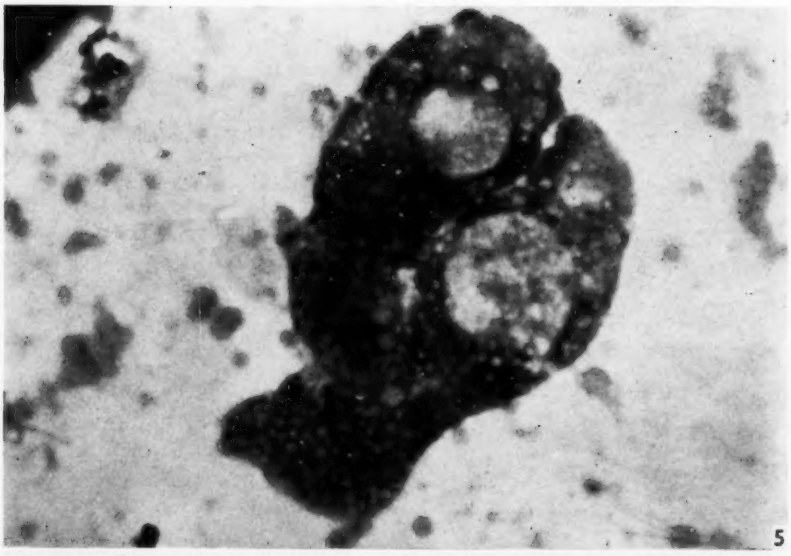
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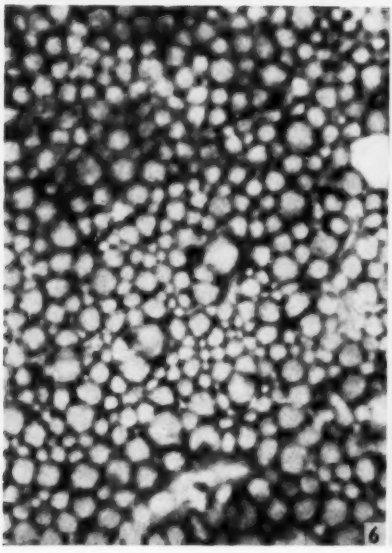
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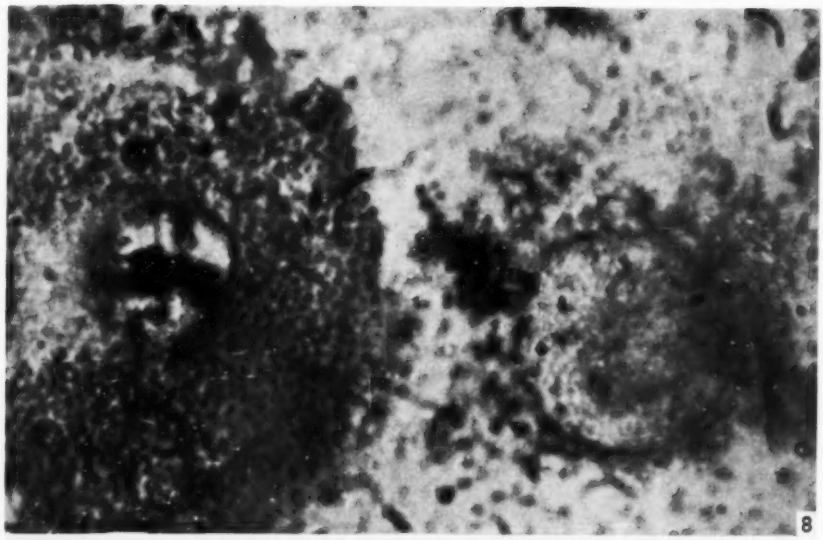

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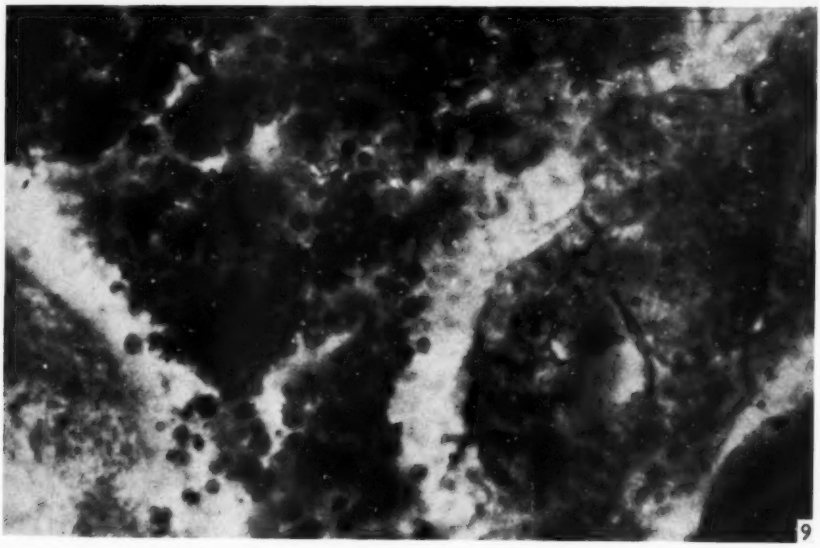

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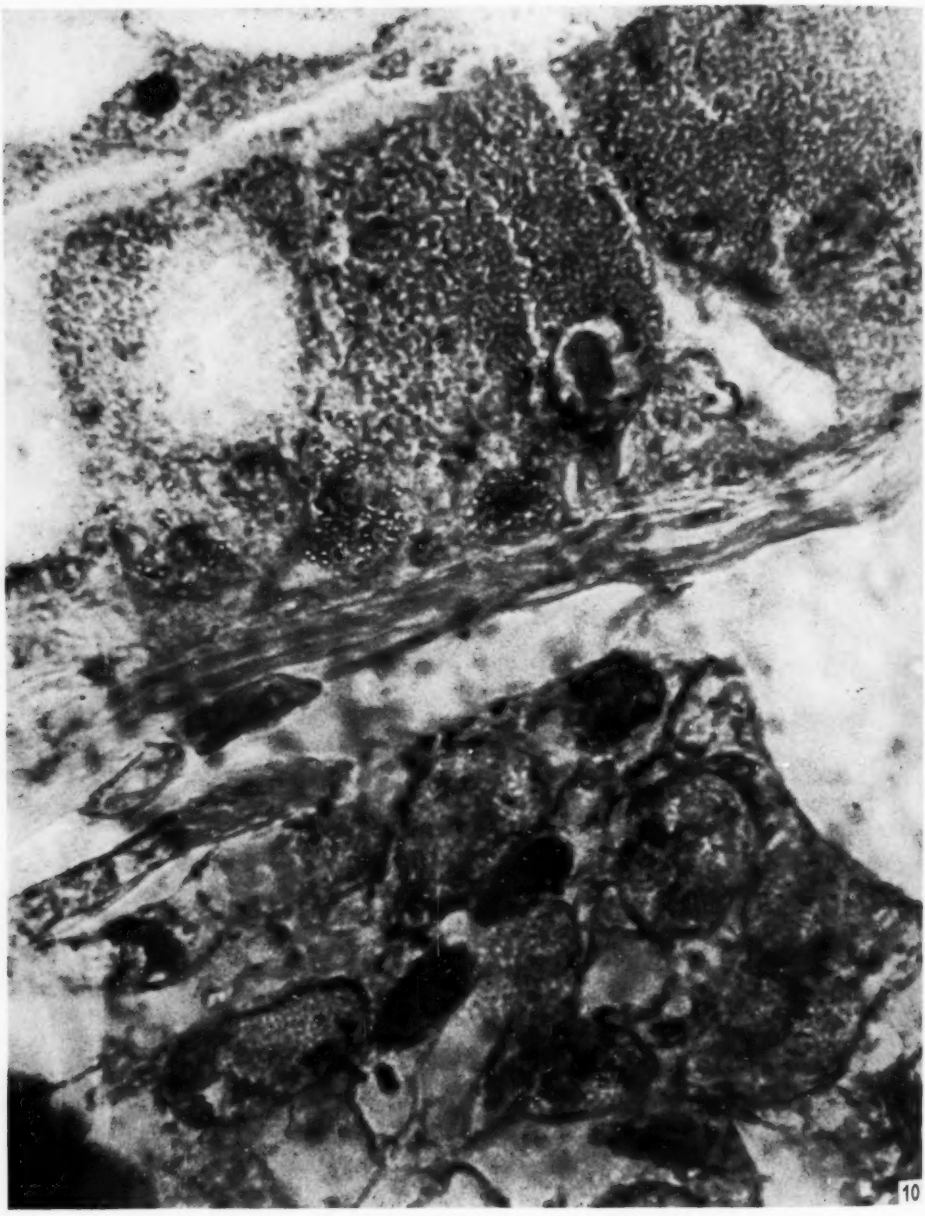
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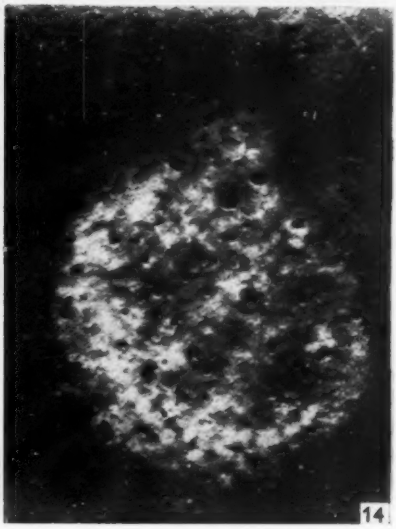
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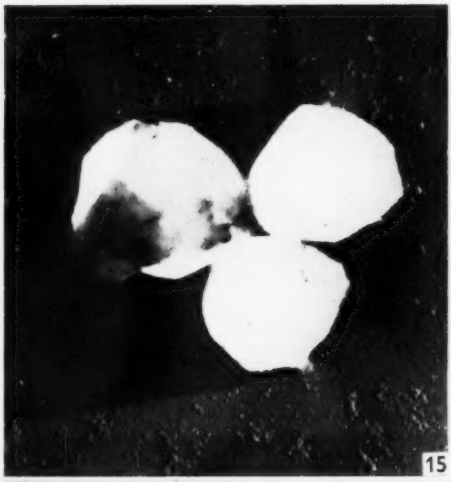
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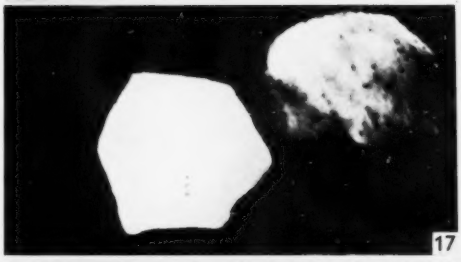
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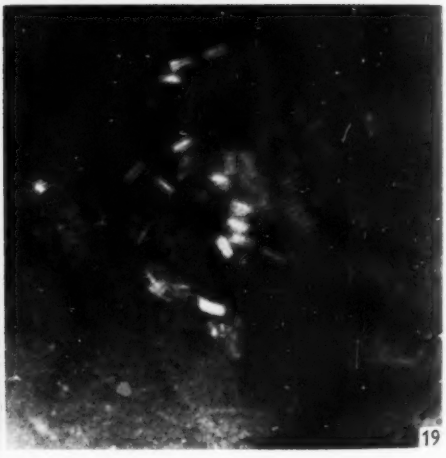
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EXPLANATION OF PLATES III–VIII

PLATE III

Fig. 1. Larvae of the small tortoiseshell butterfly *Aglais urticae*) infected with a virus from the larva of the Painted Lady butterfly (*Pyrameis cardui*). The larvae are in the condition characteristic of a nuclear polyhedrosis: note the reflexion of light from the heads of several larvae, indicating that the contents of the body are liquefied and about to run out.

Fig. 2. Similar larva to those in fig. 1; the liquefied contents of the body have now run out.

Fig. 3. A smear of the body contents of a larva of *Philosamia cynthia* affected with both types of polyhedral disease; note the non-staining nuclear polyhedra lying above masses of dark-staining cytoplasmic polyhedra. Some of these nuclear polyhedra are shown under the electron microscope in fig. 19. (Oil-immersion lens.)

Fig. 4. A smear of the body contents of *Phlogophora meticulosa* affected with both types of polyhedral disease; note the staining and non-staining polyhedra. The two kinds shown here are also shown under the electron microscope in Pl. VII, figs. 13 and 14. (Oil-immersion lens.)

PLATE IV

Fig. 5. Serial transmission of virus from *cardui* ex *urticae* ex *mori* into *mori* (silkworm): note the cytoplasmic staining polyhedra. (Oil-immersion lens.)

Fig. 6. Masses of cytoplasmic polyhedra from the gut of an infected silkworm; some of these same polyhedra are shown under the electron microscope in fig. 15. (Oil-immersion lens.)

Fig. 7. Section through part of the gut of a silkworm affected with a cytoplasmic polyhedrosis; note the large staining polyhedra above and the piece of mulberry leaf tissue in the lower right-hand corner. (Oil-immersion lens.)

PLATE V

Fig. 8. Part of a section through a larva of *cardui*, infected from *mori*. This larva had the double disease; note the nuclear non-staining polyhedra on the right and the staining cytoplasmic polyhedra on the left, with the nucleus free of polyhedra. (Oil-immersion lens.)

Fig. 9. Part of a section through a larva of *meticulosa*, infected with the double disease; note the nuclei filled with non-staining polyhedra on the right and the cytoplasmic staining type on the left. (Oil-immersion lens.)

PLATE VI

Fig. 10. Part of a section through a larva of *cardui* infected from *mori*, showing the double disease. Above is the gut tissue filled with staining polyhedra, in the cytoplasm but not in the nucleus; below is some fat body tissue showing the nuclei filled with membrane-type non-staining polyhedra. ($\frac{1}{6}$ objective.)

PLATE VII

Fig. 11. Polyhedra from *cardui* larva photographed on the electron microscope after treatment with sodium carbonate; note the membrane and virus rods. × 21,000.

Fig. 12. Polyhedra from the same larva as in fig. 11, similarly treated; note the shell-type polyhedra. × 24,000.

Fig. 13. Polyhedra from larva of *meticulosa* photographed on the electron microscope after treatment with sodium carbonate, showing membrane and virus rods. × 19,000.

Fig. 14. Polyhedra from the same larva as in fig. 13, similarly treated; note the shell-type polyhedra. × 17,000.

PLATE VIII

Fig. 15. Shell-type polyhedra from *mori* ex *cardui* treated with sodium carbonate (1 % for 1 min.) and photographed on the electron microscope: note the spherical holes in the shell. × 25,000. (Photo, R. W. G. Wyckoff.)

Fig. 16. One of the very few membrane-type polyhedra from the same larva as in fig. 15. Similar treatment except sodium carbonate 1 % for $\frac{1}{2}$ min. × 25,000. (Photo, R. W. G. Wyckoff.)

Fig. 17. Shell-type polyhedra from *meticulosa* ex *cardui* ($\frac{1}{2}$ % sodium carbonate for 5 min.). × 21,000.

Fig. 18. Membrane-type polyhedral body with virus rods from original *cardui* larva ($\frac{1}{4}$ % sodium carbonate for 2 min.). × 17,000.

Fig. 19. Membrane-type polyhedral body from *Philosamia cynthia*, showing bundles and isolated rods. × 17,000.

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